(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 June 2008 (26.06.2008)

PCT

(10) International Publication Number
WO 2008/076868 A2

(51) International Patent Classification:
G01N 33/50 (2006.01)

(21) International Application Number:
PCT/US2007/087542

(22) International Filing Date:
14 December 2007 (14.12.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/875,481 18 December 2006 (18.12.2006) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

(54) **Title:** METHODS AND COMPOSITIONS RELATED TO MODULATION OF RECEPTOR TYROSINE KINASE ORPHAN RECEPTOR-1 (ROR-1)

(57) **Abstract:** The present invention relates to the discovery of the role of tyrosine kinase orphan receptor-1 (ROR-1) and its interactions with glucose-regulated protein 78 (GRP78) and Wnt in processes such as angiogenesis and apoptosis. The invention provides diagnostic methods based on ROR-1 and GRP78, as well as methods of modulating ROR-1, antibodies specific for ROR-1, GRP78, and Wnt, and related screening methods.

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METHODS AND COMPOSITIONS RELATED TO MODULATION OF RECEPTOR TYROSINE KINASE ORPHAN RECEPTOR-1 (ROR-1)

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] Not applicable.

FIELD OF THE INVENTION

[0002] The present invention relates to generally to the area of modulation of receptor tyrosine kinase orphan receptor-1 (ROR-1). In particular, the invention relates to methods and compositions for modulating ROR-1, e.g., to produce anti-angiogenic and/or apoptotic effects, as well as to related screening methods.

BACKGROUND OF THE INVENTION

[0003] Receptor tyrosine kinase orphan receptor-1 (ROR-1) is a transmembrane tyrosine kinase protein that belongs to a family of orphan receptor kinases in mammals that consists of two members, ROR-1 and ROR-2. ROR-1 consists of five domains: IgG, frizzled, kringle, transmembrane, and a Trk-like tyrosine kinase domain. The expression of ROR-1 is high during early embryonic development; however, expression levels drop strongly around day 16, with only very low expression levels observed in adult tissues. Mice with homozygous disruption in the ROR-1 gene die within 24 hours after birth from respiratory defects. A recent publication reported that the knock-down of ROR-1 expression by RNAi led to a greater than five-fold increase in apoptosis of HeLa tumor cells, and showed that ROR-1 was among the top 4 most potent survival kinases out of the 650 kinase siRNAs tested in tumor cells responding to apoptotic stress. MacKeigan JP, Murphy LO, Blenis J., Sensitized RNAi screen of human kinases and phosphatases identifies new regulators of apoptosis and chemoresistance. Nat Cell Biol. 2005 Jun;7(6):591-600. Epub 2005 May 1.

SUMMARY OF THE INVENTION

[0004] In particular embodiments, the invention provides a method of determining whether a subject is a candidate for a therapy that includes modulating a receptor tyrosine kinase orphan receptor-1 (ROR-1). The method entails determining a level of ROR-1 in the subject, wherein the presence of an elevated level of ROR-1 indicates that the subject is a candidate for the therapy.

[0005] In exemplary embodiments, the subject is in need of anti-angiogenesis therapy and/or in need of cancer therapy. The subject can be an individual who has, or is at risk, for: a disease characterized by hypoxia, vascular disease, and/or inflammatory disease.

[0006] In certain embodiments, the method is conducted such that the presence of ROR-1 at a level that is at least twice a control level indicates that the subject is a candidate for the therapy that includes modulating a ROR-1. Additionally or alternatively, the method can entail determining a level of glucose-regulated protein 78 (GRP78) in the subject as an additional indicator of whether the subject is a candidate for the therapy, wherein the presence of an elevated level of GRP78 indicates that the subject is a candidate for the therapy.

[0007] In preferred embodiments, the method additionally comprised administering an agent that modulates ROR-1 to the subject. The agent can, for example, be a peptide, an antibody, or a small molecule. In variations of such embodiments, the agent inhibits the binding of ROR-1 to a membrane-bound protein involved in tumor survival. For example, the agent can inhibit the binding of ROR-1 to GRP78. Examples of such agents includes K5 and derivatives thereof.

[0008] The invention also provides a method of determining whether a subject is responding to a therapy that includes modulating a receptor tyrosine kinase orphan receptor-1 (ROR-1). The method entails determining the level of ROR-1 in the subject at two different timepoints, wherein the first timepoint is before or during treatment and the second timepoint is during treatment and is later than the first timepoint, and wherein a decrease in ROR-1 level at the second timepoint, relative to the first, indicates that the subject is responding to therapy.

[0009] In exemplary embodiments, the therapy is anti-angiogenesis therapy and/or cancer therapy. The subject can be an individual who has, or is at risk, for: a disease characterized by hypoxia, vascular disease, and/or inflammatory disease.

[0010] Additionally or alternatively, the method can entail determining a level of glucose-regulated protein 78 (GRP78) in the subject at the first and second timepoints as an additional indicator of whether the subject is responding to the therapy, wherein a decrease in GRP78 level at the second timepoint, relative to the first, indicates that the subject is responding to the therapy.

[0011] In any of the above-described methods, the level of ROR-1 and/or GRP78 in the subject can conveniently be determined by assaying a biological sample from the subject. The ROR-1 and/or GRP78 level assayed can, for example, be the level of ROR-1 and/or GRP78 polypeptide or the ROR-1 and/or GRP78 expression level.

[0012] The invention also provides methods of imaging a region in the body of a subject, wherein the region is affected by a condition selected from the group consisting of angiogenesis, cancer, hypoxia, vascular disease, and inflammatory disease. In certain embodiments, the method entails administering a labeled binding partner for a receptor tyrosine kinase orphan receptor-1 (ROR-1) to the subject, followed by detection of signal from the label. Additionally or alternatively, the method entails administering a labeled binding partner for a glucose-regulated protein 78 (GRP78) to the subject, followed by detection of signal from this label. Either of these exemplary methods can be carried out, for example, to image a tumor.

[0013] Other aspects of the invention include methods of testing a subject for the risk ,or presence, of a disease characterized by hypoxia. In particular embodiments, the method entails determining a level of a receptor tyrosine kinase orphan receptor-1 (ROR-1) in the subject, wherein the presence of an elevated level of ROR-1 indicates that the subject is at risk for, or has, a disease characterized by hypoxia. Alternatively, or in addition, the method entails determining a level of glucose-regulated protein 78 (GRP78) in the subject, wherein the presence of an elevated level of GRP78 indicates that the subject is at risk for, or has, a disease characterized by hypoxia.

[0014] In other embodiments, the invention provides a method of modulating a receptor tyrosine kinase orphan receptor-1 (ROR-1), wherein the method entails contacting cells, at least some of which include ROR-1 and glucose-regulated protein 78 (GRP78), with an agent that inhibits the binding of ROR-1 to GRP78, provided that the agent is not a K5 peptide or a derivative thereof. In exemplary embodiments, the agent binds to a ROR-1 kringle domain and/or the agent is a competitive inhibitor of binding of a ROR-1 kringle domain to GRP78.

[0015] The invention also provides a method of modulating a receptor tyrosine kinase orphan receptor-1 (ROR-1) to produce an apoptotic effect. The method entails contacting cells, at least some of which include ROR-1 and a Wnt, with an agent that inhibits binding of ROR-1 to Wnt, provided that the agent is not a K5 peptide or a derivative thereof. In exemplary embodiments, the agent binds to a ROR-1 frizzled domain and/or the agent is a competitive inhibitor of binding of a ROR-1 frizzled domain to Wnt. The Wnt can, for example, be Wnt11 and/or Wnt 3.

[0016] In other embodiments, the invention provides a method of modulating a receptor tyrosine kinase orphan receptor-1 (ROR-1), wherein the method entails contacting cells, at least some of which include ROR-1, with an agent that inhibits binding of the ROR-1 IgG domain to one or more ROR-1 binding partners, provided that the agent is not a K5 peptide or a derivative thereof. In exemplary embodiments, the agent binds to a ROR-1 IgG domain and/or the agent is a competitive inhibitor of binding of a ROR-1 IgG domain to the one or more ROR-1 binding partners.

[0017] The invention also provides a method of modulating a receptor tyrosine kinase orphan receptor-1 (ROR-1), wherein the method entails contacting cells, at least some of which include ROR-1, with an agent that inhibits binding of the ROR-1 kringle domain to one or more ROR-1 binding partners, provided that the agent is not a K5 peptide or a derivative thereof. In exemplary embodiments, the agent binds to a ROR-1 kringle domain and/or the agent is a competitive inhibitor of binding of a ROR-1 kringle domain to the one or more ROR-1 binding partners.

[0018] The above-described methods can be carried out, for example, to produce an anti-angiogenic and/or apoptotic effect. Agents useful in these methods can include a peptide, an antibody, and/or a small molecule. Cells useful in these methods of

modulating a ROR-1 include, but are not limited to: endothelial cells, cancer cells, and cells subject to a stress, such as, for example, hypoxic stress or chemical stress. The cells can be in vitro or in vivo. In exemplary embodiments, the cells are present in a subject in need of anti-angiogenesis therapy or a subject in need of cancer therapy. In a variation of the latter embodiment, the method additionally includes administering a cancer chemotherapeutic agent to the subject.

[0019] The invention also provides antibodies. In certain embodiments, the antibody is specific for ROR-1 and inhibits the binding of ROR-1 to GRP78. An example of an antibody of this type binds to an epitope within a ROR-1 kringle domain. Such antibodies can be produced, e.g., by immunizing an animal with a ROR-1 kringle domain or a derivative thereof and/or by a process comprising screening monoclonal antibodies or Fab fragments for binding to a ROR-1 kringle domain or a derivative thereof.

[0020] Other antibodies of the invention that are specific for ROR-1 inhibit the binding of ROR-1 to a Wnt, such as, for example, Wnt11 or Wnt3. An example of an antibody of this type binds to an epitope within a ROR-1 frizzled domain. Such antibodies can be produced, e.g., by immunizing an animal with the ROR-1 frizzled domain or a derivative thereof and/or by a process comprising screening monoclonal antibodies or Fab fragments for binding to the ROR-1 frizzled domain or a derivative thereof.

[0021] In particular embodiments, antibody is specific for: (1) GRP78 and inhibits the binding of GRP78 to ROR-1; (2) Wnt11 and inhibits binding of Wnt11 to ROR-1; and (3) Wnt3 and inhibits binding of Wnt3 to ROR-1.

[0022] The invention also provides, in certain embodiments: (1) an antibody that specifically binds the complex of ROR-1 with GRP78; and (2) an antibody that specifically binds the complex of ROR-1 with a Wnt, such as, for example, Wnt11 or Wnt3.

[0023] In particular embodiments, the antibodies of the invention are not polyclonal antibodies.

[0024] The invention also provides screening methods. In exemplary embodiments, the invention provides a method of screening for an agent that modulates receptor tyrosine kinase orphan receptor-1 (ROR-1). The method entails (a) contacting a

test agent with a ROR-1 polypeptide in the presence of a GRP78 polypeptide; and (b) detecting specific binding of the ROR-1 polypeptide with the GRP78 polypeptide relative to the the specific binding in the absence of the test agent or in the presence of a lower amount of test agent. In a variation of this method, any test agent that inhibits specific binding of the ROR-1 polypeptide with the GRP78 polypeptide is selected as an inhibitor of ROR-1.

[0025] Also provided is a method of prescreening for an agent that modulates angiogenesis and/or apoptosis. In certain embodiments, the method entails (a) contacting a test agent with a receptor tyrosine kinase orphan receptor-1 (ROR-1) polypeptide or polynucleotide; and (b) detecting specific binding of the test agent to the ROR-1 polypeptide or polynucleotide.

[0026] The invention additionally provides a method of screening for an agent that modulates angiogenesis and/or apoptosis. In particular embodiments, the method entails (a) contacting a test agent with a cell that expresses a ROR-1 polypeptide; and (b) determining the level of ROR-1 polypeptide or ROR-1 RNA, relative to the level in a cell that has not been contacted with the test agent or has been contacted with a lower amount of test agent. In a varition of this method, any test agent that reduces the level is selected as a modulator of angiogenesis.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] Figure 1. Immunohistochemical (IHC) analysis of TMA with ROR-1 antibody. Anti-ROR-1 was used to stain a TMA (Tissue MicroArray) with several tumors and matched normal tissues. A secondary goat anti-rabbit horseradish peroxidase (HRP)-conjugated antibody was used with DAB detection kit. The brown stain indicated ROR-1 protein on the cell surface.

[0028] Figure 2. IHC analysis by an ACIS machine (Chromavision, CA) for surface expression of ROR-1 on human tumor tissue. Breast, ovarian, colon, gastric, renal, and bladder cancers have high expression of ROR-1, but the matched normal tissues show significantly less ROR-1 expression.

[0029] Figure 3. A ROR-1 polyclonal antibody inhibits HT1080 tumor growth. Scid mice were inoculated with 5 $X10^6$ HT1080 tumor cells on day 0. Six hours later,

mice were injected every 3 days with either a general polyclonal control antibody or a ROR-1 antibody at 2 mg/kg/day IP. Mice showed no signs of weight loss or toxicity. A significant (p=0.02) 40% decrease in tumor volume was observed at day 11 and a 50% inhibition of tumor growth was observed at day 14 (p=0.04). Each point is an average of 10 mice.

[0030] Figure 4A-B. Amino acid sequence of human ROR-1 and recombinant kringle and IgG domains. (SEQ ID NO:1; NCBI Accession No. NM 005012). The sequence for the ROR-1 IgG domain is listed in blue from amino acids 49-146. The sequence for the ROR-1 kringle domain is listed in red. The exact amino sequences including the his-tag and thrombin cleavage sites for the ROR-1 IgG and ROR-1 kringle domain, (SED ID NO:2 and 3 respectively) are listed below the primary ROR-1 sequence. Panel A shows ROR-1 amino acids 1-500; panel B shows ROR-1 amino acids 501-938.

[0031] Figure 5A-B. Recombinant ROR-1 IgG and kringle domains. Panel A. Coomassie-stained gel of purification steps for the ROR-1 IgG domain. Lane 1: molecular weight standards; lane 2: total protein lysates; lane 3: pellet fraction after lysis; lane 4: total soluble fraction; lane 5: flow through Ni column; lane 6: purified eluted IgG fraction; lane 7: molecular weight standards. Panel B. Coomassie-stained gel of the purification steps for ROR-1 kringle domain. Lane 1: molecular weight standards; lane 2: total protein lysates; lane 3: pellet fraction after lysis; lane 4: total soluble fraction; lane 5: flow through Ni column; lane 6: purified eluted kringle fraction.

[0032] Figure 6. ROR-1 kringle domain and IgG domain blocks stimulated human microvascular endothelial cells (HMVEC) migration. Stimulated HMVEC cell chemotaxis towards vascular endothelial growth factor (VEGF; 5 ng/ml) was performed in 96-well plates for 4 hours at room temperature. Casein-AM pre-labeled HMVEC cells that had migrated to the bottom of the membrane were measure by fluorescence.

[0033] Figure 7A-C. The amino acid sequence of human plasminogen (SEQ ID NO: 6). Panel A shows plasminogen amino acids 1-270; panel B shows plasminogen amino acids 271-540; panel C shows plasminogen amino acids 541-791.

DETAILED DESCRIPTION

[0034] The work described herein provides a unique insight into the anti-apototic mechanism of ROR-1 by demonstrating that ROR-1 is an essential component of the Kringle 5 anti-tumor mechanism. Glucose-regulated protein 78 (GRP78) is expressed on the surface of activated endothelial cells and many hypoxic or chemically stressed tumor cells. During stress, we believe GRP78 chaperones ROR-1 to the cell outer membrane where it remains. The ROR-1 kringle has strong homology to the active sequence of plasminogen kringle 5 (K5). The present work shows that K5 displaces ROR-1 from its complex with GRP78, most likely based on competition with the ROR-1 kringle domain.

[0035] In addition, published data (Julia Billiard, Deana S. Way, Laura M. Seestaller-Wehr, Robert A. Moran, Annamarie Mangine and Peter V. N. Bodine, The Orphan Receptor Tyrosine Kinase Ror2 Modulates Canonical Wnt Signaling in Osteoblastic Cells. Molecular Endocrinology 19 (1): 90-101 2005) corroborates the present work that demonstrates Wnt proteins (Wnt1and Wnt3) co-immunoprecipite with ROR-1. Most Wnt proteins (Wnt1, Wnt3 and Wnt5) are anti-apoptotic and are expressed during activation of the Wnt oncogene in many tumor cells. In these tumor cells, Wnt proteins, such as Wnt 1, Wnt3, and Wnt5, prevent apoptosis through binding to a cell surface "frizzled" protein. This binding induces the assembly of an intracellular complex comprised of b-catenin binding proteins, which stabilizes b-catenin to activate survival genes. Wnt11, by contrast, is pro-apoptotic in that it competes against Wnt1, Wnt3 and Wnt5 for binding to "frizzled," but with Wnt11 bound, the complex of b-catenin binding proteins does not assemble. One possible explaination of the data is that Wnt11, and thus its pro-apoptotic function, is sequestered by its binding to the frizzled domain of ROR-1, in complex with GRP78. The binding of Wnt3 to ROR-1 may lead to increased tyrosine kinase activity through PI3K and induce cell growth and survival through a non-canonical pathway. Thus antibodies or proteins (e.g., K5), which are able to release Wnt11 or change the preference of Wnt binding proteins to ROR-1, will enhance apoptosis in Wnt-activated cells. The IgG and kringle domains are adjacent sites for interfering with Wnt binding.

[0036] In addition, the present work demonstrates that disruption of the GRP78-ROR-1 complex induces increased ROR-1 phosphorylation. Immunoprecipitation of this

activated ROR-1 co-precipitates cleaved c-Abl, which is known (Machuy N, Rajalingam K, Rudel T. Requirement of caspase-mediated cleavage of c-Abl during stress-induced apoptosis. Cell Death Differ. 2004 Mar; 11(3):290-300.) to be pro-apoptotic. Thus, attack on the cell surface complexes of ROR-1 can induce apoptosis via two mechanisms: (1) disruption of the Wnt/catenin survival pathway; and (2) activation of pro-apototic tyrosine kinases like truncated c-Abl.

Definitions

[0037] Terms used in the claims and specification are defined as set forth below unless otherwise specified.

[0038] The term "gene" refers to a polynucleotide capable of expressing a protein, including any regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence.

[0039] The term "polynucleotide" refers to a deoxyribonucleotide or ribonucleotide polymer, and unless otherwise limited, includes known analogs of natural nucleotides that can function in a similar manner to naturally occurring nucleotides. The term "polynucleotide" refers any form of DNA or RNA, including, for example, genomic DNA; complementary DNA (cDNA), which is a DNA representation of mRNA, usually obtained by reverse transcription of messenger RNA (mRNA) or amplification; DNA molecules produced synthetically or by amplification; and mRNA. The term "polynucleotide" encompasses double-stranded nucleic acid molecules, as well as single-stranded molecules. In double-stranded polynucleotides, the polynucleotide strands need not be coextensive (i.e., a double-stranded polynucleotide need not be double-stranded along the entire length of both strands).

[0040] As used herein, the term "complementary" refers to the capacity for precise pairing between two nucleotides. I.e., if a nucleotide at a given position of a polynucleotide is capable of hydrogen bonding with a nucleotide of another polynucleotide, then the two polynucleotides are considered to be complementary to one another at that position. The term "substantially complementary" describes sequences that are sufficiently complementary to one another to allow for specific hybridization under stringent hybridization conditions.

[0041] The phrase "stringent hybridization conditions" generally refers to a temperature about 5°C lower than the melting temperature (T_m) for a specific sequence at a defined ionic strength and pH. Exemplary stringent conditions suitable for achieving specific hybridization of most sequences are a temperature of at least about 60°C and a salt concentration of about 0.2 molar at pH7.

[0042] "Specific hybridization" refers to the binding of a polynucleotide to a target nucleotide sequence in the absence of substantial binding to other nucleotide sequences present in the hybridization mixture under defined stringency conditions. Those of skill in the art recognize that relaxing the stringency of the hybridization conditions allows sequence mismatches to be tolerated.

[0043] The terms "amino acid" or "amino acid residue," include naturally occurring L-amino acids or residues, unless otherwise specifically indicated. The commonly used one- and three-letter abbreviations for amino acids are used herein (Lehninger, A. L. (1975) Biochemistry, 2d ed., pp. 71-92, Worth Publishers, N. Y.). The terms "amino acid" and "amino acid residue" include D-amino acids as well as chemically modified amino acids, such as amino acid analogs, naturally occurring amino acids that are not usually incorporated into proteins, and chemically synthesized compounds having the characteristic properties of amino acids (collectively, "atypical" amino acids). For example, analogs or mimetics of phenylalanine or proline, which allow the same conformational restriction of the peptide compounds as natural Phe or Pro are included within the definition of "amino acid."

[0044] Exemplary atypical amino acids, include, for example, those described in International Publication No. WO 90/01940, as well as 2-amino adipic acid (Aad) which can be substituted for Glu and Asp; 2-aminopimelic acid (Apm), for Glu and Asp; 2-aminobutyric acid (Abu), for Met, Leu, and other aliphatic amino acids; 2-aminoheptanoic acid (Ahe), for Met, Leu, and other aliphatic amino acids; 2-aminoisobutyric acid (Aib), for Gly; cyclohexylalanine (Cha), for Val, Leu, and Ile; homoarginine (Har), for Arg and Lys; 2, 3-diaminopropionic acid (Dpr), for Lys, Arg, and His; N-ethylglycine (EtGly) for Gly, Pro, and Ala; N-ethylasparagine (EtAsn), for Asn and Gln; hydroxyllysine (Hyl), for Lys; allohydroxyllysine (Ahyl), for Lys; 3- (and 4-) hydoxyproline (3Hyp, 4Hyp), for Pro, Ser, and Thr; allo-isoleucine (Aile), for Ile, Leu, and Val; amidinophenylalanine, for Ala;

N-methylglycine (MeGly, sarcosine), for Gly, Pro, and Ala; N-methylisoleucine (MeIle), for Ile; norvaline (Nva), for Met and other aliphatic amino acids; norleucine (Nle), for Met and other aliphatic amino acids; ornithine (Orn), for Lys, Arg, and His; citrulline (Cit) and methionine sulfoxide (MSO) for Thr, Asn, and Gln; N-methylphenylalanine (MePhe), trimethylphenylalanine, halo (F, Cl, Br, and I) phenylalanine, and trifluorylphenylalanine, for Phe.

[0045] The terms "peptide," "polypeptide," and "protein" are used herein to refer a polymer of amino acids, and unless otherwise limited, include atypical amino acids that can function in a similar manner to naturally occurring amino acids. The term "peptide" is typically used with respect to relatively short polymers of amino acids, e.g., on the order of about 50 amino acids, 25 amino acids, 10 amino acids or fewer.

[0046] As used with reference to a polypeptide, the term "full-length" refers to a polypeptide having the same length as the mature wild-type polypeptide.

[0047] The term "fragment" is used herein with reference to a polypeptide or a polynucleotide to describe a portion of a larger molecule. Thus, a polypeptide fragment can lack an N-terminal portion of the larger molecule, a C-terminal portion, or both. Polypeptide fragments are also referred to herein as "peptides." A fragment of a polynucleotide can lack a 5' portion of the larger molecule, a 3' portion, or both. Nucleic acid fragments are also referred to herein as "oligonucleotides." Exemplary oligonucleotides are relatively short polynucleotides, generally shorter than 200 nucleotides, more particularly, shorter than 100 nucleotides, most particularly, shorter than 50 nucleotides. Typically, oligonucleotides are single-stranded DNA molecules.

[0048] The terms "identical" or "percent identity," in the context of two or more amino acid or nucleotide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of a sequence comparison algorithm or by visual inspection.

[0049] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequent coordinates

are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0050] Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman (1988) Proc. Natl. Acad. Sci. USA 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (see generally Ausubel et al., supra).

[0051] One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percent sequence identity. It also plots a tree or dendogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle (1987) J. Mol. Evol. 35:351-360. The method used is similar to the method described by Higgins & Sharp (1989) CABIOS 5: 151-153. The program can align up to 300 sequences, each of a maximum length of 5,000 nucleotides or amino acids. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster is then aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences are aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison and by designating the program parameters. For example, a reference sequence can be compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps.

[0052] Another example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al. (1990) J. Mol. Biol. 215: 403-410. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al, supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

[0053] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA ,90: 5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a test polynucleotide sequence is considered similar to a reference sequence if the smallest sum probability in a

comparison of the test sequence to the reference sequence is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[0054] Residues in two or more polypeptides are said to "correspond" if they are either homologous (i.e., occupying similar positions in either primary, secondary, or tertiary structure) or analogous (i.e., having the same or similar functional capacities). As is well known in the art, homologous residues can be determined by aligning the polypeptide sequences for maximum correspondence as described above.

[0055] The term "membrane-bound protein" refers to any polypeptide that is physically associated with a cell membrane. This association can be temporary (e.g., induced as part of a biological response).

[0056] As used with reference to a polypeptide, the phrase "involved in tumor survival" indicates that the polypeptide plays a role in tumor survival." Typically, such polypeptides exert some action that promotes survival of tumor cells.

[0057] As used with reference to polynucleotides or polypeptides, the term "wild-type" refers to any polynucleotide or polypeptide having a sequence present in a polynucleotide or polypeptide, respectively, from a naturally occurring organism, regardless of the source of the molecule; i.e., the term "wild-type" refers to sequence characteristics, regardless of whether the molecule is purified from a natural source; expressed recombinantly, followed by purification; or synthesized.

[0058] The term "amino acid sequence variant" refers to a polypeptide having an amino acid sequence that differs from a wild-type amino acid sequence by the addition, deletion, and/or substitution of at least one amino acid.

[0059] The term "conservative amino acid substitution" is used herein to refer to the replacement of an amino acid with a functionally equivalent amino acid. Functionally equivalent amino acids are generally similar in size and/or character (e.g., charge or hydrophobicity) to the amino acids they replace. Amino acids of similar character can be grouped as follows:

(1) hydrophobic: His, Trp, Tyr, Phe, Met, Leu, Ile, Val, Ala;

(2) neutral hydrophobic: Cys, Ser, Thr;

(3) polar: Ser, Thr, Asn, Gln;

(4) acidic/negatively charged: Asp, Glu;

(5) charged: Asp, Glu, Arg, Lys, His;

(6) basic/positively charged: Arg, Lys, His;

(7) basic: Asn, Gln, His, Lys, Arg;

(8) residues that influence chain orientation: Gly, Pro; and

(9) aromatic: Trp, Tyr, Phe, His.

[0060] The following table shows exemplary and preferred conservative amino acid substitutions.

Original Residue	Exemplary Conservative Substitution	Preferred Conservative Substitution
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln, His, Lys, Arg	Gln
Asp	Glu	Glu
Cys	Ser	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro	Pro
His	Asn, Gln, Lys, Arg	Asn
Ile	Leu, Val, Met, Ala, Phe	Leu
Leu	Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala	Leu
Pro	Gly	Gly
Ser	Thr	Thr
Thr	Ser	Ser
Trp	Tyr	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Leu, Met, Phe, Ala	Leu

[0061] As used with reference to a polypeptide or polypeptide fragment, the term "derivative" includes amino acid sequence variants as well as any other molecule that differs from a wild-type amino acid sequence by the addition, deletion, or substitution of one or more chemical groups. "Derivatives" retain at least one biological or

immunological property of a wild-type polypeptide or polypeptide fragment, such as, for example, the biological property of specific binding to a receptor and the immunological property of specific binding to an antibody.

[0062] The term "receptor tyrosine kinase orphan receptor (ROR, hereinafter)" refers to a family of tyrosine kinase receptors including ROR-1 and ROR-2. A "ROR polynucleotide" is any polynucleotide that encodes a ROR polypeptide or has at least 70% identity to a sequence derived from a ROR gene over a comparison window of at least 25 contiguous nucleotides. In alternative embodiments, this term encompasses polynucleotides sharing at least about 80, 90, 95, 96, 97, 98, or 99% identity to a sequence derived from a ROR gene.

[0063] A "ROR polypeptide" comprises an amino acid sequence that has at least about 70% identity to ROR-1 or ROR-2 over a comparison window of at least 15 contiguous amino acids. ROR polypeptides include full-length, wild-type ROR proteins, as well as ROR fragments (peptides), variants, and polypeptide derivatives. In alternative embodiments, this term encompasseses polypeptides sharing at least about 80, 90, 95, 96, 97, 98, or 99% identity to ROR-1 and/or -2.

[0064] The term "Wnt" refers to a family of conserved, cysteine-rich, secreted glycoproteins that have been found to be involved in critical aspects of early embryonic development. A "Wnt polynucleotide" is any polynucleotide that encodes a Wnt polypeptide or has at least 70% identity to a sequence derived from a Wnt gene over a comparison window of at least 25 contiguous nucleotides. In alternative embodiments, this term encompasses polynucleotides sharing at least about 80, 90, 95, 96, 97, 98, or 99% identity to a sequence derived from a Wnt gene.

[0065] A "Wnt polypeptide" comprises an amino acid sequence that has at least about 70% identity, over a comparison window of at least 15 contiguous amino acids, to any polypeptide identified in Genbank as a Wnt. Wnt polypeptides include full-length, wild-type Wnt proteins, as well as Wnt fragments (peptides), variants, and polypeptide derivatives. In alternative embodiments, this term encompasseses polypeptides sharing at least about 80, 90, 95, 96, 97, 98, or 99% identity to a Wnt.

[0066] A "GRP78 polynucleotide" is any polynucleotide that encodes a GRP polypeptide or has at least 70% identity to a sequence derived from a GRP gene over a comparison window of at least 25 contiguous nucleotides. In alternative embodiments, this term encompasses polynucleotides sharing at least about 80, 90, 95, 96, 97, 98, or 99% identity to a sequence derived from a GRP78 gene.

[0067] "GRP78 polypeptides" include full-length, wild-type GRP78 proteins, as well as GRP78 fragments (peptides), variants, and polypeptide derivatives. In alternative embodiments, this term encompasseses polypeptides sharing at least about 80, 90, 95, 96, 97, 98, or 99% identity to a GRP78.

[0068] GRP78 polynucleotides and polypeptides are described in copending, co-owned U.S. Application Nos. 10/322,853 (filed Dec. 18, 2003; incorporated by reference herein in its entirety) and 10/946,789 (filed Sep. 22, 2004; incorporated by reference herein in its entirety).

[0069] The following terms encompass polypeptides that are identified in Genbank by the following designations, as well as polypeptides that are at least about 70% identical, over a comparison window of at least 15 contiguous amino acids, to polypeptides identified in Genbank by these designations: receptor tyrosine kinase orphan receptor-1 (ROR-1), glucose-regulated protein 78 (GRP78), Wnt-3, and Wnt-11. In alternative embodiments, these terms encompass polypeptides identified in Genbank by these designations and polypeptides sharing at least about 80, 90, 95, 96, 97, 98, or 99% identity.

[0070] As used herein, the term "kringle domain" refers to a conserved sequence that folds into large loops stabilized by 3 disulfide linkages, whose conformation is defined by a number of hydrogen bonds and small pieces of an anti-parallel beta-sheet. Kringle domains are found in a varying number of copies in some plasma proteins including plasminogen, urokinase-type plasminogen activator, and prothrombin. Human ROR-1 has a kringle domain extending approximately from amino acids 310-392. The human ROR-1 sequence is shown in FIG. 4 (SEQ ID NO:1). Corresponding domains in other ROR-1 polypeptides can be determined by sequence comparisons, as described above.

[0071] As used herein, the term "kringle 5" (K5, hereinafter) refers to the region of mammalian plasminogen having three disulfide bonds which contribute to the specific three-dimensional confirmation defined by the fifth kringle domain of the mammalian plasminogen molecule. One such disulfide bond links the cysteine residues located at amino acid positions 462 and 541, a second links the cysteine residues located at amino acid positions 483 and 524, and a third links the cysteine residues located at amino acid positions 512 and 536. The amino acid sequence of a complete mammalian plasminogen molecule (the human plasminogen molecule), including its kringle 5 region, is shown in FIG. 7 (SEQ ID NO:6). The kringle 5 region has an N-terminus at about amino acid position 443 and a C-terminus at about amino acid position 546.

[0072] As used herein, the term "frizzled domain" refers to a conserved cysteine-rich domain, characterized by the sequence motif: Lys-Thr-X-X-X-Trp (SEQ ID NO:4). Human ROR-1 has a frizzled domain extending approximately from amino acids 160-297 (FIG. 4A; SEQ ID NO:5). Corresponding domains in other ROR-1 polypeptides can be determined by sequence comparisons, as described above.

[0073] As used herein, the term "IgG domain" refers to a conserved immunoglobulin-like C2-type domain. ROR-1 includes an extracellular IgG domain at approximately amino acids 49-146. Corresponding domains in other ROR-1 polypeptides can be determined by sequence comparisons, as described above.

[0074] As used herein, an "antibody" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. An antibody is said to be "specific for" a target molecule if the antibody specifically binds the target molecule. The site on the target molecule to which the antibody binds is termed an "epitope."

[0075] The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

[0076] A typical immunoglobulin (antibody) structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50 - 70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms "variable light chain (VL)" and "variable heavy chain (VH)" refer to these light and heavy chains respectively.

[0077] Antibodies include intact immunoglobulins or as a number of well-characterized fragments, which can be produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab')2, a dimer of Fab which itself is a light chain joined to VH-CH1 by a disulfide bond. The F(ab')2 may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the (Fab')2 dimer into a Fab' monomer. The Fab' monomer is essentially a Fab with part of the hinge region (see, Fundamental Immunology, W.E. Paul, ed., Raven Press, N.Y. (1993), for a more detailed description of other antibody fragments). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such Fab' fragments may be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, the term "antibody," as used herein, also includes antibody fragments either produced by the modification of whole antibodies or synthesized de novo, e.g., by using recombinant DNA methodologies. Preferred antibodies include single-chain antibodies (antibodies that exist as a single polypeptide chain), more preferably single-chain Fv antibodies (sFv or scFv) in which a variable heavy and a variable light chain are joined together (directly or through a peptide linker) to form a continuous polypeptide. The single-chain Fv antibody is a covalently linked VH-VL heterodimer, which may be expressed from a polynucleotide including VH- and VL- encoding sequences, either joined directly or joined by a peptide-encoding linker. Huston, et al. (1988) Proc. Nat. Acad. Sci. USA, 85: 5879-5883. While the VH and VL are connected to each as a single polypeptide chain, the VH and VL domains associate non-covalently. The scFv antibodies and a number of other structures convert the naturally aggregated, but chemically separated, light and heavy polypeptide chains from an antibody V region into a molecule that folds into a three dimensional structure substantially similar to the structure of an

antigen-binding site are known to those of skill in the art (see e.g., U.S. Patent Nos. 5,091,513, 5,132,405, and 4,956,778).

[0078] The term "antiserum" refers to a polyclonal antibody typically raised by immunizing an animal with an immunogen and collecting serum containing polyclonal antibodies. The serum may be subjected to one or more purification steps, including affinity purification, to produce the antiserum.

[0079] A used herein, the term "small molecule" refers to a molecule having a molecular weight of less than about 5 kilodaltons.

[0080] A "modulator" of a polypeptide (e.g., ROR-1) is either an inhibitor or an enhancer of ROR-1 action.

[0081] A "non-selective" modulator of a particular polypeptide (e.g., ROR-1) is an agent that modulates other polypeptides (e.g., ROR-2) at the concentrations typically employed for modulation of the particular polypeptide.

[0082] A "selective" modulator of a particular polypeptide significantly modulates the particular polypeptide at a concentration at which other polypeptides are not significantly modulated. Thus, a modulator can be selective for, e.g., a ROR (as opposed to other tyrosine kinases) or can be selective for a ROR subtype, such as, for example, ROR-1.

[0083] A modulator "acts directly on" a polypeptide when the modulator binds to the polypeptide, respectively.

[0084] A modulator "acts indirectly on" a polypeptide when the modulator binds to a molecule other than the polypeptide, which binding results in modulation of polypeptide function.

[0085] An "inhibitor" or "antagonist" of a polypeptide is an agent that reduces, by any mechanism, any polypeptide action, as compared to that observed in the absence (or presence of a smaller amount) of the agent. For example, an inhibitor of a receptor can affect: (1) the expression, mRNA stability, protein trafficking, modification (e.g., phosphorylation), or degradation of a receptor or of a ligand for the receptor, or (2) one or more of the normal functions of the receptor. An inhibitor of a receptor can be non-

selective or selective. Preferred inhibitors (antagonists) are generally small molecules that act directly on, and are selective for, the target receptor.

[0086] One aspect of polypeptide function that can be inhibited is the specific binding of a polypeptide to another molecule, termed a "binding partner." The term "specific binding" is defined herein as the preferential binding of binding partners to another (e.g., two polypeptides, a polypeptide and polynucleotide, or two polynucleotides) at specific sites. The term "specifically binds" indicates that the binding preference (e.g., affinity) for the target molecule/sequence is at least 2-fold, more preferably at least 5-fold, and most preferably at least 10- or 20-fold over a non-specific target molecule (e.g., a randomly generated molecule lacking the specifically recognized site(s)).

[0087] An inhibitor can inhibit the binding of a polypeptide to a binding partner "competitively" or "non-competitively." In classical competitive inhibition, the inhibitor binds the binding partner at or near the same site as the polypeptide, thus preventing the polypeptide from binding. In allosteric competitive inhibition, the inhibitor binds away from the binding site for the polypeptide, creating a conformational change in the binding partner such that the polypeptide can no longer bind to it. As used here, a "competitive inhibitor" of a particular polypeptide refers to any agent that inhibits the binding of the polypeptide by binding to the binding partner at the binding site or elsewhere. The term "competitive inhibitor" thus encompasses classical competitive inhibitors, as well as allosteric inhibitors.

[0088] The term "biological sample" refers a sample derived from an organism and includes any organ, tissue, cell, or biological fluid. A biological sample may be derived, for example, from cells or tissue cultures in vitro. Alternatively, a biological sample may be derived from a living organism or a population thereof (e.g., a population of single-cell organisms).

[0089] As used herein with respect to polypeptides, the term "elevated level" refers to a level above a "control level." These terms are typically used in the context of comparing the levels of a polypeptide in a "test" biological sample to a "control" biological sample. The test sample may be characterized by a condition not present in the control sample. In this case, the control sample will typically be "normal" with respect to this condition. Otherwise, the control sample will generally be as closely matched to the

test sample as possible. Thus, for example, the test sample may be a tumor biopsy and the control sample may be a sample from the same type of tissue at approximately the same stage of development (e.g., adult tissue is compared with adult tissue). In this example, the level of a polypeptide of interest (e.g., ROR-1) in the control sample is the "control level." A higher level of this polypeptide in the test sample indicates that the polypeptide is present at an "elevated level" in the test sample.

[0090] As used herein, the determination of a level of a polypeptide of interest can be carried out by determining the level of the polypeptide, or fragment thereof, or by determining the level of an indirect indicator of polypeptide level. Indirect measurements include measuring a gain or loss at the chromosomal locus encoding the polypeptide and/or measuring the expression of the corresponding mRNA. Chromosomal gain and/or overexpression of the corresponding mRNA indicate that the level of the encoded polypeptide is likely elevated. Conversely, chromosomal loss and/or underexpression of the corresponding mRNA indicate that the encoded polypeptide is likely reduced.

[0091] The phrases "an effective amount" and "an amount sufficient to" refer to amounts of a biologically active agent to produce an intended biological activity.

[0092] As used herein, the term "therapy" refers to any regimen aimed at the prophylaxis or treatment of any pathology and/or associated symptoms.

[0093] As used herein, the term "anti-angiogenic effect" refers to any effect that tends to inhibit the formation of new blood vessels and/or the growth of existing ones. "Anti-angiogenesis therapy" is any regimen aimed at producing an anti-angiogenic effect. Examples of diseases that are amenable to anti-angiogenesis therapy include cancer, diabetic retinopathy, and rheumatoid arthritis.

[0094] As used herein, the term "apoptotic effect" refers to any effect that tends to result in cell death.

[0095] As used herein, the term "cancer therapy" refers to any regimen for the prophylaxis or treatment of cancer.

[0096] As used herein, "a disease characterized by hypoxia" refers to any disease characterized by an insufficient supply of oxygen to any bodily tissue. This phase encompasses cerebral ischemia and ischemic heart disease.

[0097] As used herein, the term "vascular disease" refers to any disease of the vasculature and encompasses carotid artery disease, coronary artery disease, and peripheral artery disease.

[0098] As used herein, the term "inflammatory disease" refers to any disease characterized by inflammation, such as, for example, rheumatoid arthritis, inflammatory cardiovascular disease, and psoriasis.

[0099] A "test agent" is any agent that can be screened in the prescreening or screening assays of the invention. The test agent can be any suitable composition, including a small molecule, peptide, polypeptide, oligonucleotide, or polynucleotide.

I. Diagnostic Methods

A. General Approaches

[0100] In particular embodiments, the invention provides a method of determining whether a subject is a candidate for a therapy that includes modulating a receptor tyrosine kinase orphan receptor-1 (ROR-1). In certain embodiments, the method entails determining a level of ROR-1 in the subject, wherein the presence of an elevated level of ROR-1 indicates that the subject is a candidate for said therapy. In illustrative embodiments, the presence of ROR-1 at a level that is at least twice a control level indicates that the subject is a candidate for a therapy that entails modulating a ROR-1. In certain embodiments, the method alternatively or additionally entails determining a level of glucose-regulated protein 78 (GRP78) in the subject as an indicator of whether the subject is a candidate for ROR-1-based therapy, wherein the presence of an elevated level of GRP78 indicates that the subject is a candidate for ROR-1-based therapy.

[0101] Subjects that are candidates for a therapy that includes modulating a ROR-1 can alternatively or additionally be identified by measuring one or more other indications of hypoxia, e.g., by performing a standard pathology determination (histology), immunohistochemistry, or in vivo imaging. Examples of hypoxia indicators useful in this regard include: derivatives of 2-nitro-imidazole, such as 1-(2-hydroxy-3-piperidinopropyl)-2-nitro-imidazole (U.S. Patent No. 5,540,908, issued July 30, 1996, incorporated by reference herein in its entirety; U.S. Patent No. 5,674,693, issued October

7, 1997, incorporated by reference herein in its entirety); nitroaromatic compounds (U.S. Patent No. 5,843,404, issued December 1, 1998, incorporated by reference herein in its entirety); and ethylenedicysteine derivatives (U.S. Patent No. 6,692,724, issued February 17, 2004, incorporated by reference herein in its entirety; U.S. Patent No. 7,067,111, issued June 27, 2006, incorporated by reference herein in its entirety). Proteins that are up-regulated in response to hypoxia include (in addition to ROR-1 and GRP78) hypoxia-inducible factor 1 (HIF1) and p53.

[0102] Accordingly, the invention provides methods of determining whether a subject is a candidate for a therapy that includes modulating a ROR-1 that entail measuring, individually, or in any combination: ROR-1, GRP78, and one or more indicators of hypoxia.

[0103] To identify cancer patients that are candidates for a ROR-1-based therapy, the levels of one or more of the MYB transcription factor, the GTP2I (TFII-1) transcription factor, Her2/neu, and SRC can be determined independently or in conjunction with the determination of ROR-1 and/or GRP78 level(s).

[0104] The invention also provides a method of determining whether a subject is responding to a therapy that comprises modulating a ROR-1. The method entails determining the level of ROR-1 in the subject at two different timepoints, wherein the first timepoint is before or during treatment with a ROR-1 modulator, and the second timepoint is during treatment and is later than the first timepoint. A decrease in ROR-1 level at the second timepoint, relative to the first, indicates that the subject is responding to said therapy. In particular embodiments, the method alternatively or additionally includes determining a level of GRP78 in the subject at the first and second timepoints as an indicator of whether the subject is responding to the therapy, wherein a decrease in GRP78 level at the second timepoint, relative to the first, indicates that the subject is responding.

[0105] A subject's response to a therapy that includes modulating a ROR-1 can alternatively or additionally be determined by measuring one or more other indications of hypoxia, e.g., by performing a standard pathology determination (histology), immunohistochemistry, or in vivo imaging, e.g., using a hypoxia indicator, such as those described above. A decrease in a hypoxia indicator indicates that the subject is responding to the ROR-1 therapy.

[0106] Accordingly, the invention provides methods of determining whether a subject is responding to a therapy that comprises modulating a ROR-1 that entail measuring, individually, or in any combination: ROR-1, GRP78, and one or more indicators of hypoxia.

[0107] Diagnostic methods according to the invention can be carried out in vivo, e.g., using imaging techniques, or in vitro by assaying a biological sample from the subject. Suitable subjects include any animal, typically a mammal, and more typically a human.

[0108] Subjects that can be tested according to the diagnostic methods of the invention include any subject in need of, or undergoing, anti-angiogenesis therapy and/or cancer therapy. For example, the subject may be in need of, or undergoing, therapy for primary or metastatic solid tumors, including carcinomas of breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, gallbladder and bile ducts, small intestine, urinary tract (including kidney, bladder and urothelium), female genital tract, (including cervix, uterus, and ovaries as well as choriocarcinoma and gestational trophoblastic disease), male genital tract (including prostate, seminal vesicles, testes and germ cell tumors), endocrine glands (including the thyroid, adrenal, and pituitary glands), and skin, as well as hemangiomas, melanomas, sarcomas (including those arising from bone and soft tissues as well as Kaposi's sarcoma) and tumors of the brain, nerves, eyes, and meninges (including astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, and meningiomas). Suitable subjects include those who are in need of, or undergoing, therapy for solid tumors arising from hematopoietic malignancies such as leukemias (i.e. chloromas, plasmacytomas, the plaques and tumors of mycosis fungoides, and cutaneous T-cell lymphoma/leukemia) as well as lymphomas (both Hodgkin's and non-Hodgkin's lymphomas).

[0109] Subjects having, or at risk for any disease characterized by hypoxia, any type of vascular disease, and/or any inflammatory disease can also be tested to determine their suitability for, or response to, treatment based on modulating ROR-1. Thus, for example, suitable subjects include those having, or at risk for: cerebral ischemia; ischemic heart disease; carotid artery disease; coronary artery disease; myocardial angiogenesis; inflammatory cardiovascular disease; peripheral artery disease; rheumatoid, immune and

degenerative arthritis; various ocular diseases such as diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, retrolental fibroplasia, neovascular glaucoma, rubeosis, retinal neovascularization due to macular degeneration, other abnormal neovascularization conditions of the eye; skin diseases such as psoriasis; blood vessel diseases such as hemagiomas, and capillary proliferation within atherosclerotic plaques; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; and wound granulation. Other other suitable subjects include those having, or at risk for, diseases characterized by excessive or abnormal stimulation of endothelial cells, including but not limited to intestinal adhesions, Crohn's disease, atherosclerosis, scleroderma, and hypertrophic scars, i.e. keloids; as well as diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochele minalia quintosa) and ulcers (Helicobacter pylori).

[0110] When a subject is determined to be a candidate for treatment with a ROR-1 modulator, such a modulator can optionally be administered to the subject. The modulator can be an inhibitor or an enhancer of ROR-1. Subjects in need of, or undergoing, anti-angiogenesis therapy and/or cancer therapy will typically be administered an inhibitor of ROR-1, as will subjects having, or at risk for a disease characterized by hypoxia, vascular disease, and/or an inflammatory disease.

[0111] Suitable modulators include, for example, peptides, such as K5, or a derivative thereof, an antibody (e.g., such as any of those described below), or a small molecule. The modulator can act directly or indirectly on ROR-1 and can be non-selective or selective for ROR-1. In certain embodiments, the modulator inhibits the binding of ROR-1 to a membrane-bound protein involved in tumor survival. In variations of these embodiments, the membrane-bound protein is GRP78, and the modulator inhibits the binding of ROR-1 to GRP78. In an illustrative embodiment, the modulator is K5, or a derivative thereof, which inhibits the binding of ROR-1 to GRP78. K5 and related treatment methods and compositions are described in U.S. Patent No. 5,801,146 (issued Sep. 1, 1998; incorporated by reference herein in its entirety); U.S. Patent No. 5,972,896 (issued Oct. 26, 1999; incorporated by reference herein in its entirety); U.S. Patent No.5,981,484 (issued Nov. 9, 1999; incorporated by reference herein in its entirety); and U.S. Patent No. 6,251,867 (issued June 26, 2001; incorporated by reference herein in its

entirety). Methods of making K5 are described in U.S. Patent No. 6,057,122 (issued May 2, 2000; incorporated by reference herein in its entirety). K5 conjugates and fusion proteins are described in U.S. Patent No. 6,699,838 (issued Mar. 2, 2004; incorporated by reference herein in its entirety).

[0112] In other embodiments, the ROR-1 modulator inhibits the binding of ROR-1 to a Wnt (e.g., Wnt 11 and/or Wnt3). ROR-1 modulators may function by inhibiting the binding of ROR-1 to a binding partner via the any of the ROR-1 frizzled, IgG, or kringle domains. The use of ROR-1 modulators is described in greater detail below.

B. In Vivo Imaging Methods

[0113] ROR-1 and/or GRP78 levels and/or levels of indicators of hypoxia and/or cancer can be determined using in vivo imaging techniques. Scintigraphic detection is based on local physicochemical changes and is consequently superior to radiological techniques, including X-ray computed tomography, magnetic resonance imaging and ultrasonography, which rely on anatomical changes. Scintigraphic techniques can, for example, be used to detect early physiological changes, when anatomical structures have not yet been substanially altered. Scintigraphic detection techniques useful in the invention include positron emission tomography (PET) and single photon emission computed tomography (SPECT). While PET and SPECT detect distribution of radioactive probes, PET has a much greater sensitivity and resolution than SPECT. In addition, PET has the ability to measure the concentration of radioactive probes quantitatively.

[0114] Scintigraphic detection of ROR-1 and/or GRP78 levels can be carried out using a binding partner for ROR-1 and/or GRP78 and/or one or more hypoxia/cancer indicators (e.g., an antibody) that is labeled with a radioactive isotope that is readily detectable by such methods. Exemplary radioactive isotopes include ^{11}C, ^{123}I, ^{125}I, ^{73}Se, ^{76}Br, ^{77}Br, and ^{18}F. Any binding partner that can be administered to a subject and expected to the reach the site to be imaged can be employed for in vivo detection. Thus, for example, radioactively labeled anti-ROR-1 and/or anti-GRP78 antibodies can be used.

[0115] To determine, e.g., ROR-1 and/or GRP78 levels in vivo, a diagnostically effective amount of a labeled ROR-1 and/or GRP78 binding partner can be administered to an individual, followed by detection, for example, via PET or SPECT. The binding

partner can be administered to the individual by any suitable route, which may depend on whether the binding partner is to be detected anywhere in the individual's body or only at a specific site(s). Suitable routes of administration may, for example, include parenteral delivery, including delivery by intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, intranasal, and intraocular injection, as well as oral, buccal, transmucosal, transdermal, or rectal administration and administration by inhalation.

[0116] In vivo imaging can be used simply to detect the presence or absence of the target polypeptide (ROR-1, for example), to localize a site of elevated target polypeptide expression, and/or to quantify the amount of target polypeptide present at a given site in vivo. For example, this approach can be employed to identify and/or evaluate the severity of a disorder characterized by elevated ROR-1 and/or GRP78, e.g., to determine tumor size and location. In addition, this method of the invention can be used to monitor the course of such a disorder in an individual. More specifically, the size or number of sites with elevated ROR-1 and/or GRP78 levels, and/or the degree of elevation, can be measured to monitor progression of the disorder and/or determine the effectiveness of a particular therapeutic regimen aimed at ameliorating the disorder (e.g., a therapy based on modulation of ROR-1).

[0117] In vivo imaging can also be employed to detect an indication of hypoxia, e.g., using radiolabeled 2-nitroimidazole, nitroaromatic, or ethylenedicysteine compounds. Detection of any of the MYB transcription factor, the GTP2I (TFII-1) transcription factor, Her2/neu, and/or SRC can be carried out using in vivo methods as described for ROR-1 and GRP78.

C. In Vitro Methods

[0118] ROR-1, GRP78, and/or indicators of hypoxia and/or cancer can also be assayed in vitro, i.e., in a biological sample. ROR-1, GRP78, and other proteins that are upregulated in hypoxia and/or cancer can be assayed using polypeptide- and/or polynucleotide-based assays. Hypoxia can also be measured, for example, by standard histological techniques.

1. Sample Collection and Processing

[0119] Biological samples amenable to analysis using the methods of the invention include, e.g., whole blood, plasma, serum, synovial fluid, cerebrospinal fluid, bronchial lavage, ascites fluid, bone marrow aspirate, pleural effusion, and urine; tumor or other tissue from, e.g., brain, liver, lung, intestine, placenta, and uterus (e.g., tumor biopsy, needle aspirate, touch preparation, cytological smear); or any material derived therefrom, such as tissue sections, cultured cells, and cell lysates.

[0120] The sample may be pretreated as necessary by dilution in an appropriate buffer solution or concentrated, if desired. Any of a number of standard aqueous buffer solutions, employing any of a variety of buffers, such as phosphate, Tris, or the like, at physiological pH, can be used.

2. Polypeptide-Based Assays

[0121] Target polypeptides can be detected and quantified by any of a number of methods well known to those of skill in the art. These may include analytic chemical methods, such as mass spectrometry; analytic biochemical methods, such as electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, and the like; or various immunological methods such as fluid or gel precipitin reactions, immunodiffusion (single or double), immunohistochemistry, affinity chromatography, immunoelectrophoresis, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, Western blotting, and the like.

[0122] In one embodiment, polypeptide(s) are detected/quantified in an electrophoretic polypeptide separation (e.g., a 1- or 2-dimensional electrophoresis). Means of detecting polypeptides using electrophoretic techniques are well known to those of skill in the art (see generally, R. Scopes (1982) Polypeptide Purification, Springer-Verlag, N.Y.; Deutscher, (1990) Methods in Enzymology Vol. 182: Guide to Polypeptide Purification, Academic Press, Inc., N.Y.).

[0123] A variation of this embodiment utilizes a Western blot (immunoblot) analysis to detect and quantify the presence of target polypeptide(s) in the sample. This technique generally comprises separating sample polypeptides by gel electrophoresis on

the basis of molecular weight, transferring the separated polypeptides to a suitable solid support (such as a nitrocellulose filter, a nylon filter, or derivatized nylon filter), and incubating the sample with antibodies that specifically bind the target polypeptide(s). Antibodies that specifically bind to the target polypeptide(s) and may be directly labeled or alternatively may be detected subsequently using labeled antibodies (e.g., labeled sheep anti-mouse antibodies) that specifically bind to a domain of the primary antibody.

[0124] In particular embodiments, the methods of the invention can employ a "protein array" or "protein chip" format. The basic construction of such protein arrays is similar to that of nucleic acids arrays, which are described in detail below, typically utilizing a solid phase (e.g., a glass or plastic surface) spotted with an array of molecules. Each molecule is a binding partner (e.g., an antibody) designed to capture an immobilize one or more specific proteins. The immobilized protein is then detected using any suitable detection technique, including those described herein. Illustrative approaches to protein chip design that can be employed in implementing the assays of the invention include the ProteinChip by Ciphergen Biosystems Inc. (Fremont, CA), which is based on the surface-enhanced laser desorption and ionization (SELDI) process, and LabChip technology by Caliper Technologies Corp. (Mountain View, CA), which can be used with the 2100 Bioanalyzer by Agilent Technologies (Palo Alto, CA). Examples of microfluidic approaches include Biosite's (San Diego) manufacture Triage protein chip and eTag assay system by Aclara Biosciences (Hayward, CA).

[0125] Assays can be carried out on tissue samples using, e.g., an immunohistochemistry (IHC) assay. Such assays are based on the detection of binding of an antibody to an antigen of interest in the tissue sample. The methods differ primarily in the manner of detecting the antigen-antibody complex. IHC methods useful in the invention include direct method (i.e, using a labeled antibody) and indirect methods (i.e., using a labeled secondary antibody specific for the species of a primary antibody specific for the antigen). Automated immunostaining methods and reagents are described, for example, in U.S. Patent No. 5,418,138 (issued May 23, 1995). If desired, tissue arrays can be employed in the assays of the invention.

[0126] Other embodiments, can employ circulating tumor cell analysis, in which tumor cells circulating the peripheral blood of a cancer patient are obtained and analyzed,

for example, by flow cytometry or are immobilized, e.g., using a capture agent (such as an antibody) and interrogated by IHC or fluorescence in situ hybridization (FISH, see below).

i. Immunoassays

[0127] In a preferred embodiment, the target polypeptides are detected and/or quantified in the biological sample using any of a number of well-known immunoassays (see, e.g., U.S. Patent Nos. 4,366,241; 4,376,110; 4,517,288; and 4,837,168). For a general review of immunoassays, see also Methods in Cell Biology Volume 37: Antibodies in Cell Biology, Asai, ed. Academic Press, Inc. New York (1993); Basic and Clinical Immunology 7th Edition, Stites & Terr, eds. (1991).

[0128] Conventional immunoassays often utilize a "capture agent" to specifically bind to and often immobilize the analyte (e.g., a target polypeptide). In preferred embodiments, the capture agent is an antibody.

[0129] Immunoassays also typically utilize a detection agent to specifically bind to and label the binding complex formed by the capture agent and the target polypeptide. The detection agent may itself be one of the moieties making up the antibody/target polypeptide complex. Thus, the detection agent may be a labeled polypeptide or a labeled antibody that specifically recognizes the already bound target polypeptide. Alternatively, the detection agent may be an additional moiety, such as another antibody, that specifically binds to the capture agent/target polypeptide complex. Other polypeptides capable of specifically binding immunoglobulin constant regions, such as polypeptide A or polypeptide G may also be used as the detection agent. These polypeptides are normal constituents of the cell walls of streptococcal bacteria. They exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species (see, generally, Kronval, et al. (1973) J. Immunol., 111: 1401-1406, and Akerstrom (1985) J. Immunol., 135: 2589-2542).

[0130] Immunoassays for detecting the target polypeptide(s) can be either competitive or noncompetitive. Noncompetitive immunoassays include assays in which the amount of captured target polypeptide is directly measured. In competitive assays, the amount of target polypeptide in the sample can be measured indirectly by measuring the amount of an added (exogenous) polypeptide displaced (or competed away) from a

capture agent by the target polypeptide present in the sample. In one competitive assay, a known amount of, in this case, labeled target polypeptide is added to the sample, and the sample is then contacted with a capture agent. The amount of labeled target polypeptide bound to the capture agent is inversely proportional to the concentration of target polypeptide present in the sample.

[0131] The assays of this invention are scored (as positive or negative or quantity of target polypeptide) according to standard methods well known to those of skill in the art. The particular method of scoring will depend on the assay format and choice of label. For example, a Western Blot assay can be scored by visualizing the colored product produced by the enzymatic label. A clearly visible colored band or spot at the correct molecular weight is scored as a positive result, while the absence of a clearly visible spot or band is scored as a negative. The intensity of the band or spot can provide a quantitative measure of target polypeptide concentration.

[0132] Antibodies useful in these immunoassays include polyclonal and monoclonal antibodies, which can be produced, for example, as described below.

[0133] For embodiments of the invention that employ a solid phase as a support for the capture agent, the solid phase can be any suitable material with sufficient surface affinity to bind a capture agent. Useful solid supports include: natural and synthetic polymeric carbohydrates; crosslinked, or substituted derivatives, such as agar, agarose, cross-linked alginic acid, substituted and cross-linked guar gums, cellulose esters, especially with nitric acid and carboxylic acids, mixed cellulose esters, and cellulose ethers; natural polymers containing nitrogen, such as proteins and derivatives, including, but not limited to cross-linked or modified gelatins; natural hydrocarbon polymers, such as latex and rubber; synthetic polymers, such as vinyl polymers, including polyethylene, polypropylene, polystyrene, polyvinylchloride, polyvinylacetate and its partially hydrolyzed derivatives, polyacrylamides, polymethacrylates, copolymers and terpolymers of the above polycondensates, such as polyesters, polyamides, and other polymers, such as polyurethanes or polyepoxides; inorganic materials such as sulfates or carbonates of alkaline earth metals and magnesium, including barium sulfate, calcium sulfate, calcium carbonate, silicates of alkali and alkaline earth metals, aluminum and magnesium; and aluminum or silicon oxides or hydrates, such as clays, alumina, talc, kaolin, zeolite, silica

gel, or glass (these materials may be used as filters with the above polymeric materials); and mixtures or copolymers of the above classes, such as graft copolymers obtained by initializing polymerization of synthetic polymers on a pre-existing natural polymer. All of these materials may be used in suitable shapes, such as films, sheets, tubes, particulates, or plates, or they may be coated onto, bonded, or laminated to appropriate inert carriers, such as paper, glass, plastic films, fabrics, or the like.

[0134] Nitrocellulose has excellent absorption and adsorption qualities for a wide variety of reagents including monoclonal antibodies. Nylon also possesses similar characteristics and also is suitable.

[0135] Preferred solid phase materials for flow-through assay devices include filter paper such as a porous fiberglass material or other fiber matrix materials. The thickness of such material is not critical and will be a matter of choice, largely based upon the properties of the sample or analyte being assayed, such as the fluidity of the biological sample.

[0136] Alternatively, the solid phase can constitute microparticles. Microparticles useful in the invention can be selected by one skilled in the art from any suitable type of particulate material and include those composed of polystyrene, polymethylacrylate, polypropylene, latex, polytetrafluoroethylene, polyacrylonitrile, polycarbonate, or similar materials. Further, the microparticles can be magnetic or paramagnetic microparticles, so as to facilitate manipulation of the microparticle within a magnetic field.

[0137] Microparticles can be suspended in the mixture of soluble reagents and biological sample or can be retained and immobilized by a support material. In the latter case, the microparticles on or in the support material are not capable of substantial movement to positions elsewhere within the support material. Alternatively, the microparticles can be separated from suspension in the mixture of soluble reagents and biological sample by sedimentation or centrifugation. When the microparticles are magnetic or paramagnetic the microparticles can be separated from suspension in the mixture of soluble reagents and biological sample by a magnetic field.

[0138] The methods of the present invention can be adapted for use in systems that utilize microparticle technology including automated and semi-automated systems

wherein the solid phase comprises a microparticle. Such systems include those described in U.S. Patent Nos. 5,089,424 and 5,244,630, which correspond to published EPO App. Nos. EP 0 425 633 and EP 0 424 634, respectively, and U.S. Patent No. 5,006,309.

[0139] In particular embodiments, the solid phase includes one or more electrodes. Capture agent(s) can be affixed, directly or indirectly, to the electrode(s). In one embodiment, for example, capture agents can be affixed to magnetic or paramagnetic microparticles, which are then positioned in the vicinity of the electrode surface using a magnet. Systems in which one or more electrodes serve as the solid phase are useful where detection is based on electrochemical interactions. Exemplary systems of this type are described, for example, in U.S. Patent No. 6,887,714 (issued May 3, 2005). The basic method is described further below with respect to electrochemical detection.

[0140] The capture agent can be attached to the solid phase by adsorption, where it is retained by hydrophobic forces. Alternatively, the surface of the solid phase can be activated by chemical processes that cause covalent linkage of the capture agent to the support.

[0141] To change or enhance the intrinsic charge of the solid phase, a charged substance can be coated directly onto the solid phase. Ion capture procedures for immobilizing an immobilizable reaction complex with a negatively charged polymer, described in EP Publication No. 0326100, and U.S.App. No. 375,029 (EP Publication No. 0406473), can be employed according to the present invention to affect a fast solution-phase immunochemical reaction. In these procedures, an immobilizable immune complex is separated from the rest of the reaction mixture by ionic interactions between the negatively charged polyanion/immune complex and the previously treated, positively charged matrix and detected by using any of a number of signal-generating systems, including, e.g., chemiluminescent systems, as described in U.S. App. No. 921,979, corresponding to EPO Publication No. 0 273,115.

[0142] If the solid phase is silicon or glass, the surface must generally be activated prior to attaching the specific binding partner. Activated silane compounds such as triethoxy amino propyl silane (available from Sigma Chemical Co., St. Louis, Mo.), triethoxy vinyl silane (Aldrich Chemical Co., Milwaukee, Wis.), and (3-mercapto-propyl)-trimethoxy silane (Sigma Chemical Co., St. Louis, Mo.) can be used to introduce reactive

groups such as amino-, vinyl, and thiol, respectively. Such activated surfaces can be used to link the capture directly (in the cases of amino or thiol), or the activated surface can be further reacted with linkers such as glutaraldehyde, bis (succinimidyl) suberate, SPPD 9 succinimidyl 3-[2-pyridyldithio] propionate), SMCC (succinimidyl-4-[Nmaleimidomethyl] cyclohexane-1-carboxylate), SIAB (succinimidyl [4iodoacetyl] aminobenzoate), and SMPB (succinimidyl 4-[1maleimidophenyl] butyrate) to separate the capture agent from the surface. Vinyl groups can be oxidized to provide a means for covalent attachment. Vinyl groups can also be used as an anchor for the polymerization of various polymers such as poly-acrylic acid, which can provide multiple attachment points for specific capture agents. Amino groups can be reacted with oxidized dextrans of various molecular weights to provide hydrophilic linkers of different size and capacity. Examples of oxidizable dextrans include Dextran T-40 (molecular weight 40,000 daltons), Dextran T-110 (molecular weight 110,000 daltons), Dextran T-500 (molecular weight 500,000 daltons), Dextran T-2M (molecular weight 2,000,000 daltons) (all of which are available from Pharmacia, Piscataway, N.J.), or Ficoll (molecular weight 70,000 daltons; available from Sigma Chemical Co., St. Louis, Mo.). Additionally, polyelectrolyte interactions can be used to immobilize a specific capture agent on a solid phase using techniques and chemistries described U.S. App. No. 150,278, filed Jan. 29, 1988, and U.S. App. No. 375,029, filed Jul. 7, 1989, each of which is incorporated herein by reference.

[0143] Other considerations affecting the choice of solid phase include the ability to minimize non-specific binding of labeled entities and compatibility with the labeling system employed. For example, solid phases used with fluorescent labels should have sufficiently low background fluorescence to allow signal detection.

[0144] Following attachment of a specific capture agent, the surface of the solid support may be further treated with materials such as serum, proteins, or other blocking agents to minimize non-specific binding.

ii. Polypeptide Detection

[0145] Detectable labels suitable for use in the detection agents of the present invention include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical, or chemical means. Useful labels in the

present invention include magnetic beads (e.g., Dynabeads™), fluorescent dyes (e.g., fluorescein, Texas Red, rhodamine, green fluorescent protein, and the like, see, e.g., Molecular Probes, Eugene, Oregon, USA), chemiluminescent compounds such as acridinium (e.g., acridinium-9-carboxamide), phenanthridinium, dioxetanes, luminol and the like, radiolabels (e.g., ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P), catalysts such as enzymes (e.g., horse radish peroxidase, alkaline phosphatase, beta-galactosidase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold (e.g., gold particles in the 40 -80 nm diameter size range scatter green light with high efficiency) or colored glass or plastic (e.g., polystyrene, polypropylene, latex, etc.) beads. Patents teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

[0146] The label can be attached to the detection agent prior to, or during, or after contact with the biological sample. So-called "direct labels" are detectable labels that are directly attached to or incorporated into detection agents prior to use in the assay. Direct labels can be attached to or incorporated into detection agents by any of a number of means well known to those of skill in the art.

[0147] In contrast, so-called "indirect labels" typically bind to the detection agent at some point during the assay. Often, the indirect label binds to a moiety that is attached to or incorporated into the detection agent prior to use. Thus, for example, an antibody used as a detection agent (a "detection antibody") can be biotinylated before use in an assay. During the assay, an avidin-conjugated fluorophore can bind the biotin-bearing detection agent, to provide a label that is easily detected. In another example of indirect labeling, polypeptides capable of specifically binding immunoglobulin constant regions, such as polypeptide A or polypeptide G, can also be used as labels for detection antibodies.

[0148] Some labels useful in the invention may require the use of an indicator reagent to produce a detectable signal. In an ELISA, for example, an enzyme label (e.g., beta-galactosidase) will require the addition of a substrate (e.g., X-gal) to produce a detectable signal.

iii. Exemplary Formats

[0149] In an exemplary embodiment, a fluorescent label is employed in a fluorescence polarization immunoassay (FPIA) according to the invention. Generally, fluorescent polarization techniques are based on the principle that a fluorescent label, when excited by plane-polarized light of a characteristic wavelength, will emit light at another characteristic wavelength (i.e., fluorescence) that retains a degree of the polarization relative to the incident light that is inversely related to the rate of rotation of the label in a given medium. As a consequence of this property, a label with constrained rotation, such as one bound to another solution component with a relatively lower rate of rotation, will retain a relatively greater degree of polarization of emitted light than when free in solution.

[0150] This technique can be employed in immunoassays according to the invention, for example, by selecting reagents such that binding of the fluorescently labeled entities forms a complex sufficiently different in size such that a change in the intensity light emitted in a given plane can be detected.

[0151] Fluorophores useful in FPIA include fluorescein, aminofluorescein, carboxyfluorescein, and the like, preferably 5 and 6-aminomethylfluorescein, 5 and 6-aminofluorescein, 6-carboxyfluorescein, 5-carboxyfluorescein, thioureafluorescein, and methoxytriazinolyl-aminofluorescein, and similar fluorescent derivatives. Examples of commercially available automated instruments with which fluorescence polarization assays can be conducted include: IMx.RTM. system, TDx.RTM. system, and TDxFLx.TM. system (all available from Abbott Laboratories, Abbott Park, Ill.).

[0152] The use of scanning probe microscopy (SPM) for immunoassays also is a technology to which the immunoassay methods of the present invention are easily adaptable. In SPM, in particular in atomic force microscopy, the capture agent is affixed to a solid phase having a surface suitable for scanning. The capture agent can, for example, be adsorbed to a plastic or metal surface. Alternatively, the capture agent can be covalently attached to, e.g., derivatized plastic, metal, silicon, or glass according to methods known to those of ordinary skill in the art. Following attachment of the capture agent, the biological sample is contacted with the solid phase, and a scanning probe microscope is used to detect and quantify solid phase-affixed complexes. The use of SPM

eliminates the need for labels which are typically employed in immunoassay systems. Such a system is described in U.S. App. No. 662,147, which is incorporated herein by reference.

[0153] Immunoassays according to the invention can also be carried out using a MicroElectroMechanical System (MEMS). MEMS are microscopic structures integrated onto silicon that combine mechanical, optical, and fluidic elements with electronics, allowing convenient detection of an analyte of interest. An exemplary MEMS device suitable for use in the invention is the Protiveris' multicantilever array. This array is based on chemo-mechanical actuation of specially designed silicon microcantilevers and subsequent optical detection of the microcantilever deflections. When coated on one side with a binding partner, a microcantilever will bend when it is exposed to a solution containing the complementary molecule. This bending is caused by the change in the surface energy due to the binding event. Optical detection of the degree of bending (deflection) allows measurement of the amount of complementary molecule bound to the microcantilever.

[0154] In other embodiments, immunoassays according to the invention are carried out using electrochemical detection. A basic procedure for electrochemical detection has been described by Heineman and coworkers. This entailed immobilization of a primary antibody (Ab, rat-anti mouse IgG), followed by exposure to a sequence of solutions containing the antigen (Ag, mouse IgG), the secondary antibody conjugated to an enzyme label (AP-Ab, rat anti mouse IgG and alkaline phosphatase), and p-aminophenyl phosphate (PAPP). The AP converts PAPP to p-aminophenol (PAP_R, the "R" is intended to distinguish the reduced form from the oxidized form, PAP_O, the quinoneimine), which is electrochemically reversible at potentials that do not interfere with reduction of oxygen and water at pH 9.0, where AP exhibits optimum activity. PAP_R does not cause electrode fouling, unlike phenol whose precursor, phenylphosphate, is often used as the enzyme substrate. Although PAP_R undergoes air and light oxidation, these are easily prevented on small scales and short time frames. Picomole detection limits for PAP_R and femtogram detection limits for IgG achieved in microelectrochemical immunoassays using PAPP volumes ranging from 20 .mu.l to 360 μL have been reported previously. In capillary immunoassays with electrochemical detection, the lowest detection limit reported thus far

is 3000 molecules of mouse IgG using a volume of 70 μL and a 30 min or 25 min assay time.

[0155] Various electrochemical detection systems are described in U.S. Patent Nos. 7,045,364 (issued May 16, 2006; incorporated herein by reference), 7,045,310 (issued May 16, 2006; incorporated herein by reference), 6,887,714 (issued May 3, 2005; incorporated herein by reference), 6,682,648 (issued January 27, 2004; incorporated herein by reference); 6,670,115 (issued December 30, 2003; incorporated herein by reference).

[0156] In particular embodiments, useful, for example, for simultaneously assaying multiple analytes in one biological sample, the solid phase can include a plurality different capture agents. Examples of this format include microfluidic devices and capillary arrays, containing different capture agents at different locations along a channel or capillary, respectively, and microarrays, which typically contain different capture agents arranged in a matrix of spots ("target elements") on a surface of a solid support. In particular embodiments, each different capture agent can be affixed to a different electrode, which can, for example, be formed on a surface of a solid support, in a channel of a microfluidic device, or in a capillary. Multiplex formats can, but need not, employ a plurality of different detection agents, wherein each agent is used for the detection of a target polypeptide.

3. Polynucleotide Based Assays

[0157] Changes in chromosomal copy numbr or in expression level can be detected by measuring changes in genomic DNA or in mRNA and/or a polynucleotide derived from the mRNA (e.g., reverse-transcribed cDNA, etc.). Furthermore, changes in expression level can also be detected by measuring changes in the degree of methylation of the relevant gene.

i. Amplification-Based Assays

[0158] In particular embodiments, amplification-based assays can be used to detect, and optionally quantify, polynucleotides of interest. In such amplification-based assays, the ROR-1 and/or GRP78 polynucleotides in the sample act as template(s) in an amplification reaction carried out with a nucleic acid primer that contains a detectable

label or component of a labeling system. Suitable amplification methods include, but are not limited to, polymerase chain reaction (PCR); reverse-transcription PCR (RT-PCR); ligase chain reaction (LCR) (see Wu and Wallace (1989) Genomics 4: 560, Landegren et al. (1988) Science 241: 1077, and Barringer et al. (1990) Gene 89: 117; transcription amplification (Kwoh et al. (1989) Proc. Natl. Acad. Sci. USA 86: 1173), self-sustained sequence replication (Guatelli et al. (1990) Proc. Nat. Acad. Sci. USA 87: 1874); dot PCR, and linker adapter PCR, etc.

[0159] To determine the level of a ROR-1 and/or GRP78 polynucleotide and/or of other indicators of hypoxia and/or cancer, any of a number of well known "quantitative" amplification methods can be employed. Quantitative PCR (also called "real-time" PCR) generally involves simultaneously co-amplifying a known quantity of a control sequence using the same primers. This provides an internal standard that may be used to calibrate the PCR reaction. Detailed protocols for quantitative PCR are provided in PCR Protocols, A Guide to Methods and Applications, Innis et al., Academic Press, Inc. N.Y., (1990).

ii. Hybridization-Based Assays

[0160] In certain embodiments, hybrization-based assays can be used to detect, and optionally quantify, target polynucleotides. Nucleic acid hybridization simply involves contacting a nucleic acid probe with sample polynucleotides under conditions where the probe and its complementary target nucleotide sequence can form stable hybrid duplexes through complementary base pairing. The nucleic acids that do not form hybrid duplexes are then washed away leaving the hybridized nucleic acids to be detected, typically through detection of an attached detectable label or component of a labeling system. Methods of detecting and/or quantifying polynucleotides using nucleic acid hybridization techniques are known to those of skill in the art (see Sambrook et al. supra). Hybridization techniques are generally described in Hames and Higgins (1985) Nucleic Acid Hybridization, A Practical Approach, IRL Press; Gall and Pardue (1969) Proc. Natl. Acad. Sci. USA 63: 378-383; and John et al. (1969) Nature 223: 582-587. Methods of optimizing hybridization conditions are described, e.g., in Tijssen (1993) Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 24: Hybridization With Nucleic Acid Probes, Elsevier, N.Y.).

[0161] Nucleic acid probes used for detection of the target polynucleotides can be full-length or less than the full-length of these polynucleotides. Shorter probes are generally empirically tested for specificity. Preferably, nucleic acid probes are at least about 15, and more preferably about 20 bases or longer, in length. (See Sambrook et al. for methods of selecting nucleic acid probe sequences for use in nucleic acid hybridization.) Visualization of the hybridized probes allows the qualitative determination of the presence or absence of the target polynucleotide of interest, and standard methods (such as, e.g., densitometry where the nucleic acid probe is radioactively labeled) can be used to quantify the level of the target polynucleotide.)

[0162] A variety of additional nucleic acid hybridization formats are known to those skilled in the art. Standard formats include sandwich assays and competition or displacement assays. Sandwich assays are commercially useful hybridization assays for detecting or isolating polynucleotides. Such assays utilize a "capture" nucleic acid covalently immobilized to a solid support and a labeled "signal" nucleic acid in solution. The sample provides the target polynucleotide. The capture nucleic acid and signal nucleic acid each hybridize with the target polynucleotide to form a "sandwich" hybridization complex.

[0163] In particular embodiments, the methods of the invention can be utilized in array-based hybridization formats. In an array format, a large number of different hybridization reactions can be run essentially "in parallel." This provides rapid, essentially simultaneous, evaluation of a number of hybridizations in a single experiment. Methods of performing hybridization reactions in array-based formats are well known to those of skill in the art (see, e.g., Pastinen (1997) Genome Res. 7: 606-614; Jackson (1996) Nature Biotechnology 14:1685; Chee (1995) Science 274: 610; WO 96/17958, Pinkel et al. (1998) Nature Genetics 20: 207-211).

[0164] Arrays, particularly nucleic acid arrays, can be produced according to a wide variety of standard methods. For example, in a simple embodiment, "low-density" arrays can simply be produced by spotting (e.g., by hand, using a pipette) different nucleic acids at different locations on a solid support (e.g., a glass surface, a membrane, etc.). This simple spotting approach has been automated to produce high-density spotted microarrays. For example, U.S. Patent No. 5,807,522 describes the use of an automated

system that taps a microcapillary against a surface to deposit a small volume of a biological sample. The process is repeated to generate high-density arrays. Arrays can also be produced using oligonucleotide synthesis technology. Thus, for example, U.S. Patent No. 5,143,854 and PCT Patent Publication Nos. WO 90/15070 and 92/10092 teach the use of light-directed combinatorial synthesis of high-density oligonucleotide microarrays. Synthesis of high-density arrays is also described in U.S. Patents 5,744,305; 5,800,992; and 5,445,934.

[0165] In an illustrative embodiment, the arrays used in this invention contain "probe" nucleic acids (e.g., oligonucleotide, cDNA, or genomic DNA). These probes are then hybridized respectively with their "target" nucleotide sequence(s) present in polynucleotides derived from a biological sample. Alternatively, the format can be reversed, such that polynucleotides from different samples are arrayed and this array is then probed with one or more probes, which can be differentially labeled.

[0166] Many methods for immobilizing nucleic acids on a variety of solid phases are known in the art. A wide variety of organic and inorganic polymers, as well as other materials, both natural and synthetic, can be employed as the material for the solid phase. Illustrative solid phases include, e.g., nitrocellulose, nylon, glass, quartz, diazotized membranes (paper or nylon), silicones, polyformaldehyde, cellulose, and cellulose acetate. In addition, plastics such as polyethylene, polypropylene, polystyrene, and the like can be used. Other materials that can be employed include paper, ceramics, metals, metalloids, semiconductive materials, and the like. In addition, substances that form gels can be used. Such materials include, e.g., proteins (e.g., gelatins), lipopolysaccharides, silicates, agarose and polyacrylamides. Where the solid phase is porous, various pore sizes may be employed depending upon the nature of the system.

[0167] In preparing the surface, a plurality of different materials may be employed, particularly as laminates, to obtain various properties. For example, proteins (e.g., bovine serum albumin) or mixtures of macromolecules (e.g., Denhardt's solution) can be employed to avoid non-specific binding, simplify covalent conjugation, and/or enhance signal detection. If covalent bonding between a compound and the surface is desired, the surface will usually be polyfunctional or be capable of being polyfunctionalized. Functional groups that may be present on the surface and used for linking can include

carboxylic acids, aldehydes, amino groups, cyano groups, ethylenic groups, hydroxyl groups, mercapto groups and the like. The manner of linking a wide variety of compounds to various surfaces is well known and is amply illustrated in the literature.

[0168] Arrays can be made up of target elements of various sizes, ranging from about 1 mm diameter down to about 1 μm. Relatively simple approaches capable of quantitative fluorescent imaging of 1 cm^2 areas have been described that permit acquisition of data from a large number of target elements in a single image (see, e.g., Wittrup (1994) Cytometry 16:206-213, Pinkel et al. (1998) Nature Genetics 20: 207-211).

[0169] In certain embodiments, methods of the invention employ a DNA microarray containing probes for one or more of the following loci: 1p32-1p31 (the locus of ROR-1), 9q33-9q34.1 (the locus of GRP78), 6q22-6q23 (the locus of the MYB transcription factor), 7q11.23 (the locus of GTP2I [TFII-1] transcription factor), 17q21.1 (the locus of Her2/neu), and 20q12-q13 (the locus of SRC), allowing detection of gain at these loci as an indication of an increase in the level of the encoded polypeptide. In other embodiments, the methods of the invention employ an expression microarray, which measures the mRNA corresponding to one or more of of these genes, allowing detection of overexpression.

[0170] Hybridization assays according to the invention can be carried out using a MicroElectroMechanical System (MEMS), such as the Protiveris' multicantilever array.

[0171] Tissue samples can be analyzed by in situ hybridization. Whole mount in-situ hybridization is used to view the location of polynucleotides in situ (i.e. in their native location in the cell or nucleus). To detect RNA, the tissue is gently fixed so as not to disrupt the double-stranded DNA. Tissues are then hybridized to a labeled nucleic acid probe that is complimentary (or anti-sense) to the RNA of interest. To detect DNA, chromosomes are briefly exposed to high pH to disrupt the DNA base pairs followed by hybridization with an appropriate nucleic acid probe. In situ hybridization can identify the sequence of interest concentrated in the cells containing it. In situ hybridization can also identify the type and fraction of the cells in a heterogeneous cell population containing the sequence of interest. DNA and RNA can be detected with the same assay reagents. Amplification methods can be employed to enhance signal, or peptide nucleic acids or morpholino compounds, e.g., can be utilized in in situ hybridization methods to detect

targets without the need for amplification. Probes can be labeled with any of the labels described below. When flourescent labels are used, the method is termed "FISH" (fluorescence in situ hybridization). If increased signal is desired in a FISH assay, mutiple fluorophores can be used to increase signal and, thus, sensitivity of the method. Various methods of FISH are known, including a one-step method using multiple oligonucleotides or the conventional multi-step method. It is within the scope of the present invention that these types of methods can be automated by various means including flow cytometry and image analysis.

[0172] In particular embodiments, in situ hybridization can be employed to detect aneuploidy of chromosome 1 and/or chomosomal gain at 1p32-1p31 can be detected as an indication of elevated ROR-1. Aneuploidy of chromosome 9 and/or chromosomal gain at 9q33-9q34 can be detected as an indication of elevated GRP78. The detection of these chromosomal abnormalities is useful in diagnosing cancers and identifying those cancers that are likely to respond to treatment based on modulating ROR-1. Such cancers can alternatively, or additionally, be identified by detecting gain at one or more of the following loci: 6q22-6q23 (the locus of the MYB transcription factor), 7q11.23 (the locus of GTP2I [TFII-1] transcription factor), 17q21.1 (the locus of Her2/neu), and 20q12-q13 (the locus of SRC).

iii. Methylation-Based Assays

[0173] For gene-specific methylation analysis, a large number of techniques have been developed. Most early studies used methylation sensitive restriction enzymes to digest DNA followed by Southern detection or PCR amplification. Recently, bisulfite reaction based methods have become very popular such as methylation specific PCR (MSP), bisulfite genomic sequencing PCR.

[0174] As methylation is associated with transcriptional silencing, the detection of a reduced level of methylation of the gene (e.g., at the promoter) indicates increased expression of ROR-1 and/or GRP78 and/or one or more other protein indicators of hypoxia or cancer. Thus, the degree of methylation serves as an additional indicator of the level of ROR-1, GRP78, and/or one or more hypoxia or cancer indicators.

iv. Polynucleotide Detection

[0175] Target polynucleotides are detected in the above-described polynucleotide-based assays by means of a detectable label. Any of the labels discussed above can be used in the polynucleotide-based assays of the invention. The label may be added to a probe or primer or sample polynucleotides prior to, or after, the hybridization or amplification. Direct labels are directly attached to or incorporated into the labeled polynucleotide prior to conducting the assay. In indirect labeling, one of the polynucleotides in the hybrid duplex carries a component to which the detectable label binds. Thus, for example, a probe or primer can be biotinylated before hybridization. After hybridization, an avidin-conjugated fluorophore can bind the biotin-bearing hybrid duplexes, providing a label that is easily detected. For a detailed review of methods of the labeling and detection of polynucleotides, see Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 24: Hybridization With Nucleic Acid Probes, P. Tijssen, ed. Elsevier, N.Y., (1993)).

[0176] The sensitivity of the hybridization assays can be enhanced through use of a polynucleotide amplification system that multiplies the target polynucleotide being detected. Examples of such systems include the polymerase chain reaction (PCR) system and the ligase chain reaction (LCR) system. Other methods recently described in the art are the nucleic acid sequence-based amplification (NASBAO, Cangene, Mississauga, Ontario) and Q Beta Replicase systems.

[0177] In a preferred embodiment, suitable for used in amplification-based assays of the invention, a primer contains two fluorescent dyes, a "reporter dye" and a "quencher dye." When intact, the primer produces very low levels of fluorescence because of the quencher dye effect. When the primer is cleaved or degraded (e.g., by exonuclease activity of a polymerase, see below), the reporter dye fluoresces and is detected by a suitable fluorescent detection system. Amplification by a number of techniques (PCR, RT-PCR, RCA, or other amplification method) is performed using a suitable DNA polymerase with both polymerase and exonuclease activity (e.g., Taq DNA polymerase). This polymerase synthesizes new DNA strands and, in the process, degrades the labeled primer, resulting in an increase in fluorescence. Commercially available fluorescent

detection systems of this type include the ABI Prism® Systems 7000, 7700, or 7900 (TaqMan®) from Applied Biosystems or the LightCycler® System from Roche.

4. Test Kits

[0178] The invention also provides test kits for assaying biological samples for one or more target polypeptides and/or polynucleotides. Test kits according to the invention include one or more reagents useful for practicing one or more assays according to the invention. A test kit generally includes a package with one or more containers holding the reagents, as one or more separate compositions or, optionally, as admixture where the compatibility of the reagents will allow. The test kit can also include other material(s), which may be desirable from a user standpoint, such as a buffer(s), a diluent(s), a standard(s), and/or any other material useful in sample processing, washing, or conducting any other step of the assay.

[0179] Test kits according to the invention preferably include instructions for carrying out one or more of the immunoassays of the invention. Instructions included in kits of the invention can be affixed to packaging material or can be included as a package insert. While the instructions are typically written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this invention. Such media include, but are not limited to, electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. As used herein, the term "instructions" can include the address of an internet site that provides the instructions.

II. Methods of Modulating ROR-1

A. General Approaches

[0180] The invention also provides methods of modulating ROR-1. In certain embodiments, such a method entails contacting cells, at least some of which include ROR-1 and glucose-regulated protein 78 (GRP78), with an agent that inhibits the binding of ROR-1 to GRP78, provided that the agent is not a K5 peptide or a derivative thereof. The agent can be one that inhibits ROR-1-GRP78 binding by inhibiting binding via a ROR-1 kringle domain. In related embodiments, the agent inhibits the binding of the ROR-1

kringle domain to one or more other binding partners. The agent can bind to the ROR-1 kringle domain or can competitively inhibit binding of the ROR-1 kringle domain to GRP78 or the other binding partner, e.g., by binding the ROR-1 binding site on GRP78 or the other binding partner, respectively.

[0181] In particular embodiments, the invention provides methods of modulating a ROR-1 to produce an apoptotic effect. Such methods entail contacting cells, at least some of which comprise ROR-1 and a Wnt, with an agent that inhibits binding of ROR-1 to Wnt, provided that the agent is not a K5 peptide or a derivative thereof. The agent can be one that inhibits ROR-1-Wnt binding by inhibiting binding via a ROR-1 frizzled domain. Thus, the agent can bind to the ROR-1 frizzled domain or can competitively inhibit binding of the ROR-1 frizzled domain to Wnt, e.g., by binding the ROR-1 binding site on Wnt. The Wnt can, for example, be Wnt11 and/or Wnt3.

[0182] In other embodiments, ROR-1 can be modulated by contacting cells, at least some of which comprise ROR-1, with an agent that inhibits binding of the ROR-1 IgG domain to one or more ROR-1 binding partners, provided that the agent is not a K5 peptide or a derivative thereof. The agent can bind to the ROR-1 IgG domain or can competitively inhibit binding of the ROR-1 IgG domain, e.g., by binding the ROR-1 binding site on the binding partner(s).

[0183] In certain embodiments, the ROR-1 is modulated to produce an anti-angiogenic effect. Alternatively or additionally, the modulation of ROR-1 can produce an apoptotic effect.

B. Cells/Subjects

[0184] Any cell that includes ROR-1 and GRP78, Wnt (e.g., Wnt11 and/or Wnt3), and/or another ROR-1 binging partner that binds ROR-1 via its kringle, frizzled, or IgG domain can be employed in the methods of modulating ROR-1. The cell is typically, although not necessarily, one that expresses these polypeptides endogenously. The method generally employs animal cells, typically cells from vertebrates, preferably from birds or mammals, more preferably from animals having research or commercial value or value as pets, such as mice, rats, guinea pigs, rabbits, cats, dogs, chickens, pigs, sheep,

goats, cows, horses, as well as monkeys and other primates. Human cells can be employed.

[0185] In particular embodiments, the method employs endothelial cells, cancer cells, and/or cells subject to stress, such as, e.g., a hypoxic stress or a chemical stress.

[0186] The cells are generally contacted with modulator under physiological conditions. The duration of contact with the modulator can vary, depending on the particular application of the method. The duration of contact can range from minutes to days or longer. For research applications, the modulator is typically contacted with cells for, e.g., about 30 mins.; or about 1, about 3, about 6, or about 12 hours; or about 1, about 2, about 5, about 10, or about 15 days; or about 5, about 10, or about 15 weeks; or about 2, about 4, about 6, about 8, or about 10 months; or about 1, about 2, about 3, about 4, or about 5 years.

[0187] Contact of the modulator with cells can be achieved directly, i.e., by administering a composition containing the modulator to the cells, or indirectly, e.g., by administering a composition containing a polynucleotide encoding a modulator polypeptide to the cells. In the latter embodiment, this administration results in the introduction of the polynucleotide into one or more cells and the subsequent expression of the polypeptide in an amount sufficient to modulate ROR-1. In one embodiment, a composition containing a polynucleotide encoding an anti-ROR-1 antibody is administered to the cells.

[0188] This method can be carried out in vitro, i.e., in cells or tissues that are not part of an organism, or in vivo, in cells that are part of an organism. In one embodiment, cells are contacted in vitro in with an effective amount of an modulator (or a polynucleotide encoding the modulator).

[0189] Alternatively, cells can be contacted in vivo with a modulator by administering a composition containing the modulator (or a polynucleotide encoding the modulator) directly to a subject. Suitable subjects include all of those describe above, with respect to the method of determining whether a subject is a candidate for ROR-1 therapy. For example, the subject can be one in need of anti-angiogenesis therapy and/or

cancer therapy; where the subject is a cancer patient, the method can additionally include administering a cancer chemotherapeutic agent to the subject.

C. ROR-1 Inhibitors

[0190] In particular embodiments, ROR-1 inhibitors reduce specific binding of ROR-1 to a binding partner, such that the binding observed in the presence of the inhibitor is less than that observed in the absence of inhibitor (or in the presence of a lower amount of inhibitor). Suitable inhibitors disrupt receptor-ROR-1 and/or GRP78 binding, for example, by binding to the binding domain of one of the binding partners; binding near the domain and sterically hindering access to the domain; binding one of the binding partners and inducing a conformational change in the domain. In various embodiments, the inhibitor reduces binding by at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, and 95 percent, as determined by a binding assay.

[0191] In particular embodiments, the ROR-1 inhibitor is a polypeptide, such as, for example, peptide, or an antibody. In other embodiments, the inhibitor is a small molecule.

D. Formulations

[0192] For administration to cells, tissues, or subjects, ROR-1 modulators are typically combined with a pharmaceutically acceptable carrier (excipient), such as are described in Remington's Pharmaceutical Sciences (1980) 16th editions, Osol, ed., 1980. Pharmaceutically acceptable carriers can contain one or more physiologically acceptable compound(s) that act, for example, to stabilize the composition or to increase or decrease the absorption of the ROR-1 modulator(s). A pharmaceutically acceptable carrier suitable for use in the invention is non-toxic to cells, tissues, or subjects at the dosages employed, and can include a buffer (such as a phosphate buffer, citrate buffer, and buffers made from other organic acids), an antioxidant (e.g., ascorbic acid), a low-molecular weight (less than about 10 residues) peptide, a polypeptide (such as serum albumin, gelatin, and an immunoglobulin), a hydrophilic polymer (such as polyvinylpyrrolidone), an amino acid (such as glycine, glutamine, asparagine, arginine, and/or lysine), a monosaccharide, a disaccharide, and/or other carbohydrates (including glucose, mannose, and dextrins), a chelating agent (e.g., ethylenediaminetetratacetic acid [EDTA]), a sugar alcohol (such as

mannitol and sorbitol), a salt-forming counterion (e.g., sodium), and/or an anionic surfactant (such as Tween™, Pluronics™, and PEG). In one embodiment, the pharmaceutically acceptable carrier is an aqueous pH-buffered solution.

[0193] Other pharmaceutically acceptable compounds include wetting agents, emulsifying agents, dispersing agents or preservatives that are particularly useful for preventing the growth or action of microorganisms. Various preservatives are well known and include, for example, phenol and ascorbic acid. One skilled in the art would appreciate that the choice of pharmaceutically acceptable carrier(s), including a physiologically acceptable compound depends, for example, on the route of administration of the ROR-1 modulator(s) and on the particular physio-chemical characteristics of the ROR-1 modulator(s).

[0194] Pharmaceutical compositions of the invention can be stored in any standard form, including, e.g., an aqueous solution or a lyophilized cake. Such compositions are typically sterile when administered to subjects. Sterilization of an aqueous solution is readily accomplished by filtration through a sterile filtration membrane. If the composition is stored in lyophilized form, the composition can be filtered before or after lyophilization and reconstitution.

E. Administration

[0195] For in vitro applications, cells are contacted with an modulator of the invention simply by adding the modulator or the polynucleotide encoding the modulator directly to the medium of cultured cells or directly to tissues.

[0196] Methods for in vivo administration do not differ from known methods for administering drugs or therapeutic polypeptides, peptides, or polynucleotides encoding them. Suitable routes of administration include, for example, topical, intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, or intralesional routes. Pharmaceutical compositions of the invention can be administered continuously by infusion, by bolus injection, or, where the compositions are sustained-release preparations, by methods appropriate for the particular preparation.

[0197] In certain embodiments, ROR-1 can be inhibited by introducing a nucleic acid construct that expresses an intrabody into the target cells. An intrabody is an

intracellular antibody, in this case, capable of recognizing and binding to ROR-1 or a ROR-1 binding partner. The intrabody is expressed by an "antibody cassette" containing: (1) a sufficient number of nucleotides encoding the portion of an antibody capable of binding to the target polypeptide operably linked to (2) a promoter that will permit expression of the antibody in the cell(s) of interest. The construct encoding the intrabody is delivered to the cell where the antibody is expressed intracellularly and binds to the target polypeptide, thereby disrupting the target from its normal action.

[0198] In a preferred embodiment, the "intrabody gene" of the antibody cassette includes a cDNA encoding heavy chain variable (V_H) and light chain variable (V_L) domains of an antibody which can be connected at the DNA level by an appropriate oligonucleotide linker, which on translation, forms a single peptide (referred to as a single chain variable fragment, "sFv") capable of binding to a target polypeptide. The intrabody gene preferably does not encode an operable secretory sequence, and thus the expressed antibody remains within the cell.

[0199] Antibodies suitable for use/expression as intrabodies in the methods of this invention can be readily produced by a variety of methods. Such methods include, but are not limited to, traditional methods of raising antibodies, which can be modified to form single chain antibodies, or screening of, e.g., phage display libraries to select for antibodies showing high specificity and/or avidity for ROR-1 or one of its binding partners.

[0200] The antibody cassette is delivered to the cell by any means suitable for introducing polynucleotides into cells. A preferred delivery system is described in U.S. Patent 6,004,940. Methods of making and using intrabodies are described in detail in U.S. Patents 6,072,036, 6,004,940, and 5,965,371.

F. Dose

[0201] The dose of modulator is sufficient to modulate ROR-1 without significant toxicity. For in vivo applications, the dose of modulator depends, for example, upon the therapeutic objectives, the route of administration, and the condition of the subjected. Accordingly, it is necessary for the clinician to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Generally, the clinician

begins with a low dose and increases the dosage until the desired therapeutic effect is achieved. Starting doses for a given modulator can be extrapolated from in vitro data.

III. Antibodies

[0202] The present invention also provides antibodies.

A. Binding Characteristics

[0203] In particular embodiments, an antibody of the invention is specific for ROR-1 and inhibits the binding of ROR-1 to GRP78 and is not a polyclonal antibody. In variations of such embodiments, the antibody binds to an epitope within a ROR-1 kringle domain.

[0204] Other ROR-1-specific antibodies of the invention inhibit the binding of ROR-1 to a Wnt and are not polyclonal. The Wnt can be, for example, Wnt 11 and/or Wnt3. In variations of such embodiments, the antibody binds to an epitope within a ROR-1 frizzled domain.

[0205] In other embodiments, an antibody of the invention is specific for GRP78 and inhibits the binding of GRP78 to ROR-1.

[0206] The invention also provides, in certain embodiments, an antibody specific for a Wnt, wherein the antibody inhibits binding of the Wnt to ROR-1. In illustrative embodiments, the Wnt is Wnt 11 or Wnt3.

[0207] The invention additionally provides, in certain embodiments, an antibody that specifically binds the complex of ROR-1 with GRP78, wherein the antibody is not a polyclonal antibody. In other embodiments, the antibody specifically binds the complex of receptor ROR-1 with a Wnt, wherein the antibody is not a polyclonal antibody. In variations of such embodiments, the Wnt is Wnt11 or Wnt3.

B. Antibody Production

[0208] The invention encompasses polyclonal and monoclonal anti-ROR-1, anti-Wnt (e.g., anti-Wnt11 and anti-Wnt3), and anti-GRP78 antibodies. Polyclonal antibodies

are raised by injecting (e.g., subcutaneous or intramuscular injection) antigenic polypeptides into a suitable non-human mammal (e.g., a mouse or a rabbit). Generally, the polypeptide used as the antigen should induce production of high titers of antibody with relatively high affinity for ROR-1, Wnt (e.g, Wnt11 or Wnt3), or GRP78, and, in particular, for any domains of interest (e.g., kringle, frizzled, or IgG).

[0209] If desired, the immunizing polypeptide may be conjugated to a carrier protein by conjugation using techniques that are well known in the art. Commonly used carriers include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid. The conjugate is then used to immunize the animal.

[0210] The antibodies are then obtained from blood samples taken from the animal. The techniques used to produce polyclonal antibodies are extensively described in the literature (see, e.g., Methods of Enzymology, "Production of Antisera With Small Doses of Immunogen: Multiple Intradermal Injections", Langone, et al. eds. (Acad. Press, 1981)). Polyclonal antibodies produced by the animals can be further purified, for example, by binding to and elution from a matrix to which the peptide to which the antibodies were raised is bound (e.g., the ROR-1 kringle, frizzled, or IgG domains). Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal, as well as monoclonal, antibodies see, for example, Coligan, et al. (1991) Unit 9, Current Protocols in Immunology, Wiley Interscience).

[0211] For many applications, monoclonal antibodies are preferred. The general method used for production of hybridomas secreting mAbs is well known (Kohler and Milstein (1975) Nature, 256:495). Briefly, as described by Kohler and Milstein, the technique entailed isolating lymphocytes from regional draining lymph nodes of five separate cancer patients with either melanoma, teratocarcinoma or cancer of the cervix, glioma or lung, (where samples were obtained from surgical specimens), pooling the cells, and fusing the cells with SHFP-1. Hybridomas were screened for production of antibody which bound to cancer cell lines. Confirmation of specificity among mAb's can be accomplished using relatively routine screening techniques (such as the enzyme-linked immunosorbent assay, or "ELISA") to determine the elementary reaction pattern of the mAb of interest.

[0212] As used herein, the term "antibody" encompasses antigen-binding antibody fragments, e.g., single chain antibodies (scFv or others), which can be produced/selected using phage display technology. The ability to express antibody fragments on the surface of viruses that infect bacteria (bacteriophage or phage) makes it possible to isolate a single binding antibody fragment, e.g., from a library of greater than 10^{10} nonbinding clones. To express antibody fragments on the surface of phage (phage display), an antibody fragment gene is inserted into the gene encoding a phage surface protein (e.g., pIII) and the antibody fragment-pIII fusion protein is displayed on the phage surface (McCafferty et al. (1990) Nature, 348: 552-554; Hoogenboom et al. (1991) Nucleic Acids Res. 19: 4133-4137).

[0213] Since the antibody fragments on the surface of the phage are functional, phage bearing antigen binding antibody fragments can be separated from non-binding phage by antigen affinity chromatography (McCafferty et al. (1990) Nature, 348: 552-554). Depending on the affinity of the antibody fragment, enrichment factors of 20-fold - 1,000,000-fold are obtained for a single round of affinity selection. By infecting bacteria with the eluted phage, however, more phage can be grown and subjected to another round of selection. In this way, an enrichment of 1000-fold in one round can become 1,000,000-fold in two rounds of selection (McCafferty et al. (1990) Nature, 348: 552-554). Thus even when enrichments are low (Marks et al. (1991) J. Mol. Biol. 222: 581-597), multiple rounds of affinity selection can lead to the isolation of rare phage. Since selection of the phage antibody library on antigen results in enrichment, the majority of clones bind antigen after as few as three to four rounds of selection. Thus only a relatively small number of clones (several hundred) need to be analyzed for binding to antigen.

[0214] Human antibodies can be produced without prior immunization by displaying very large and diverse V-gene repertoires on phage (Marks et al. (1991) J. Mol. Biol. 222: 581-597). In one embodiment natural VH and VL repertoires present in human peripheral blood lymphocytes are isolated from unimmunized donors by PCR. The V-gene repertoires were spliced together at random using PCR to create a scFv gene repertoire which is was cloned into a phage vector to create a library of 30 million phage antibodies (Id.). From this single "naïve" phage antibody library, binding antibody fragments have been isolated against more than 17 different antigens, including haptens, polysaccharides and proteins (Marks et al. (1991) J. Mol. Biol. 222: 581-597; Marks et al.

(1993). Bio/Technology. 10: 779-783; Griffiths et al. (1993) EMBO J. 12: 725-734; Clackson et al. (1991) Nature. 352: 624-628). Antibodies have been produced against self proteins, including human thyroglobulin, immunoglobulin, tumor necrosis factor and CEA (Griffiths et al. (1993) EMBO J. 12: 725-734). It is also possible to isolate antibodies against cell surface antigens by selecting directly on intact cells. The antibody fragments are highly specific for the antigen used for selection and have affinities in the 1 nM to 100 nM range (Marks et al. (1991) J. Mol. Biol. 222: 581-597; Griffiths et al. (1993) EMBO J. 12: 725-734). Larger phage antibody libraries result in the isolation of more antibodies of higher binding affinity to a greater proportion of antigens.

IV. Screening Methods

[0215] The invention provides screening methods based on the interactions of ROR-1 with is binding partners, in particular, GRP78 and Wnt (e.g., Wnt11 and Wnt3). The prescreening/screening methods of the invention are generally, although not necessarily, carried out in vitro. Accordingly, screening assays are generally carried out, for example, using purified or partially purified components (e.g., ROR-1, GRP78, and/or Wnt polypeptides or polynucleotides), in cell lysates, in cultured cells, or in a biological sample.

A. Methods of Screening for Agents that Modulate ROR-1

[0216] In particular embodiments, the invention provides a method of screening for an agent that modulates ROR-1. The method entails contacting a test agent with a ROR-1 polypeptide in the presence of a binding partner polypeptide (e.g., ROR-1, GRP78, and/or Wnt), and detecting specific binding of the ROR-1 polypeptide with the binding partner polypeptide relative to the specific binding in the absence of the test agent or in the presence of a lower amount of test agent. Specific binding can be detected in a binding assay, such as any of those described above with respect to detection of ROR-1 and/or GRP78 polypeptides. In illustrative embodiments, any test agent that inhibits specific binding of the ROR-1 polypeptide with the binding partner polypeptide is selected as an inhibitor of ROR-1.

B. Methods of Screening for Agents that Modulate Angiogenesis or Apoptosis

[0217] The role of ROR-1 and GRP78 polypeptides in angiogenesis makes the ROR-1-GRP78 interaction an attractive target for agents that modulate angiogenesis. Similarly, the role of ROR-1 and Wnt (e.g., Wnt11 and Wnt3) in apoptosis makes the ROR-1-Wnt interaction an attractive target for agents that modulate this process. Accordingly, the invention provides prescreening and screening methods aimed at identifying agents that either inhibit or enhance angiogenesis and apoptosis.

1. Prescreening Based on Binding to ROR Polypeptides or Polynucleotides

[0218] The prescreening methods are based on screening test agents for specific binding to a ROR-1 polypeptide or polynucleotide. In certain embodiments, a prescreening method of the invention entails contacting a test agent with a ROR-1 polypeptide. Specific binding of the test agent to the ROR-1 polypeptide is then detected. Suitable ROR-1 polypeptides include an amino acid sequence that has at least about 70% identity to ROR-1 over a comparison window of at least 15 contiguous amino acids. Agents that specifically bind to ROR-1 polypeptides have the potential to decrease or increase ROR-1 function.

[0219] Agents that specifically bind to ROR-1 polynucleotides have the potential to decrease or increase the expression of ROR-1 polypeptides, which can inhibit or enhance, respectively, ROR-1 function. Thus, in alternative embodiments, to screen for agents that affect ROR-1 expression, the test agent can be contacted with a polynucleotide encoding the ROR-1 polypeptide, followed by detection of specific binding of the test agent to the ROR-1 polynucleotide.

[0220] In illustrative embodiments, any test agent that specifically binds the ROR-1 polypeptide or polynucleotide is selected as a possible modulator of ROR-1, and therefore, angiogenesis and/or apoptosis.

2. Screening Based on Levels of ROR-1 Polypeptide or RNA

[0221] Test agents, including, for example, those identified in a prescreening assay of the invention can also be screened to determine whether the test agent affects the levels

of ROR-1 polypeptide or RNA. Agents that reduce these levels can potentially inhibit ROR-1 function, and thereby inhibit angiogenesis and/or promote apoptosis. Conversely, agents that increase these levels can potentially enhance ROR-1 function, and thereby enhance angiogeneis and/or inhibit apoptosis.

[0222] Accordingly, the invention provides a method of screening for an agent that inhibits or enhances ROR-1 function in which a test agent is contacted with a cell. The cell is one that expresses a ROR-1 polypeptide. After contact with the test agent, the level of ROR-1 polypeptide or RNA is determined to identify any test agents that affect these levels.

[0223] Cells useful in this screening method include those described above with respect to methods of modulating ROR-1. Cells that naturally express ROR-1 are typically, although not necessarily, employed in this screening method. ROR-1 polypeptide and polynucleotide can be detected as described above.

[0224] In a preferred embodiment, generally involving the screening of a large number of test agents, the screening method includes the recordation of any test agent that induces a difference in the level of ROR-1 polypeptide or RNA in a database of agents that modulate angiogenesis and/or apoptosis.

C. Test Agent Databases

[0225] The term "database" refers to a means for recording and retrieving information. In preferred embodiments, the database also provides means for sorting and/or searching the stored information. The database can employ any convenient medium including, but not limited to, paper systems, card systems, mechanical systems, electronic systems, optical systems, magnetic systems or combinations thereof. Preferred databases include electronic (e.g. computer-based) databases. Computer systems for use in storage and manipulation of databases are well known to those of skill in the art and include, but are not limited to "personal computer systems," mainframe systems, distributed nodes on an inter- or intra-net, data or databases stored in specialized hardware (e.g. in microchips), and the like.

D. Selected Test Agent Compositions

[0226] Screening methods of the invention optionally include combining the a selected test agent with a carrier, preferably pharmaceutically acceptable carrier. Generally, the concentration of the agent is sufficient to modulate ROR-1 function when the composition is contacted with a cell. This concentration will vary, depending on the particular modulator and specific application for which the composition is intended.

EXAMPLES

[0227] The following examples are offered to illustrate, but not to limit, the claimed invention.

Example 1

ROR-1 Expression on Tumor Tissues

[0228] ROR-1 is highly expressed on many tumor tissues (breast cancer, prostate cancer, ovarian, melanoma, and glioma) but rarely expressed on adult normal tissue (Figures 1-2).

Example 2

Polyclonal Antibody Specific for ROR-1 Inhibits Proliferation in Tumor Cell Lines

[0229] Studies with a polyclonal antibody raised to the N-terminal peptide from ROR-1 and purified with this same peptide show that binding to ROR-1 leads to tumor cell apoptosis in vitro. Table 1 shows potent dose-response inhibition of cellular proliferation in six out of the seven cell lines tested with the anti-ROR-1. Normal human kidney and renal cells displayed no proliferation inhibition by the ROR-1 antibody.

Table 1. Proliferation results for anti-ROR-1 on various cell lines.

		% Inhibition of Proliferation (Anti-ROR-1)*		
Cell Line	**Tumor type**	**0.1 μg/ml**	**1 μg/ml**	**10 μg/ml**
EaHy	Endothelial cell	28 + 2	40 +3	79 + 5
PC-3	Prostate carcinoma	8 + 5	15 + 4	40 + 9
D54	Glioma	0 + 5	12 + 6	56 + 5
HT1080	Fibrosarcoma	5 + 4	17 + 2	44 + 10
Calu 6	Lung carcinoma	7 + 2	21 + 3	30 + 4
MDA231	Breast carcinoma	2 + 4	13 + 2	46 + 6
HT29	Colon carcinoma	0 + 2	10 + 8	0 + 10
Kidney fibroblasts	Normal cells	0 + 8	0 + 10	0 + 6

* Cells were plated in 96 well plates at approximately 3000 cells per well overnight. The media was removed, and fresh complete media was added with the anti-ROR-1. The plates were incubated for 72 hours at 37C in 5%CO2. After 72 hours, cell number was determined by with a CCK-F kit.

Example 3

Polyclonal Antibody Specific for ROR-1 Slows Tumor Growth

[0230] To validate the importance of the ROR-1 IgG domain, a commercially available polyclonal ROR-1 N-terminal antibody was used at 1 mg/kg/day in vivo in an HT1080 flank tumor model. The antibody to ROR-1 significantly slowed the growth rate of an HT1080 tumor by 50% (Figure 3).

Example 4

Fragments of ROR-1 Inhibit Tumor Cell Proliferation and Endothelial Cell Migration

[0231] Several different ROR-1 domains were prepared and evaluated for their ability to inhibit tumor cell and endothelial cell function. A domain coding amino acids 310-392 (kringle) of ROR-1 (Figure 4) was obtained from Daudi mRNA (Clontech) by reverse transcription (RT)-PCR using an RT PCR kit (Roche Molecular Biochemicals). The fragment was inserted into the pETas plasmid (Invitrogen) for expression (Studier, F.W. et al. (1986) J. Mol. Biol. 189: 113-130). A second domain coding amino acids 49-146 (IgG) of ROR-1 (Figure 5) was obtained as described above and inserted into the pETas plasmid.

[0232] The ROR-1 protein domains were expressed in *Escherichia c*oli BL21(DE3) cells which were grown in M9 medium. The ROR-1 soluble protein domains were purified by Ni^{2+}-affinity chromatography. The amino-terminal His tag was removed by cleavage with biotinylated thrombin according to the manufacturer's protocol (Novagen). The thrombin was removed by adding Streptavidin Agarose (Novagen) to the reaction mix, and the cleaved His tag and any uncleaved protein were removed by passing the mixture over another pre-equilibrated Ni^{2+} column. For the kringle and IgG domains, the soluble fraction after cell lysis was loaded on a Q-Sepharose column, washed with a buffer containing 25 mM Tris·HCl (pH 8.0) and 1 mM DTT, and eluted with a linear gradient of 0-1 M NaCl. The eluted fractions containing the ROR-1 domains were concentrated and loaded on a Superdex 75 gel-filtration column pre-equilibrated with a buffer containing 25 mM Tris·HCl (pH 8.0). The ROR-1 kringle and IgG domains were dialysed overnight against PBS (pH 7.4). Purified proteins were considered greater than 95% pure by gel electrophoresis (Figure 5) and mass spec analysis.

[0233] The effect of the ROR-1 kringle domain and the ROR-1 IgG domain on endothelial cell migration was determined by using 96-well plates with a cellulose membrane between the upper and the lower chambers (Neuroprobe). Human microvascular endothelial cells (HMVEC) were starved of growth factors overnight, labeled with fluorescent calcein AM (50-100 nmol/L), plated into a 96-well migration chamber (2.9 x 104 cells per well) and stimulated to migrate with vascular endothelial growth factor (VEGF; 5 ng/mL) added to the bottom side of the chamber. After 4 hours, migrated cells were measured by fluorescence (Frevert CW, Wong VA, Goodman RB, Goodwin R, Martin TR. Rapid fluorescence-based measurement of neutrophil migration in vitro . J Immunol Methods 1998;213:41–52). In a concentration-dependent manner, the kringle domain and the IgG domain from ROR-1 reduced VEGF-stimulated migration of HMVEC (Figure 6). The IC50 for the kringle domain of ROR-1 was 5-10 nM, whereas the IC50 for the IgG domain of ROR-1 was 0.1 nM. These results suggest ROR-1 plays a critical role in the VEGF-stimulated migration of endothelial cells.

[0234] The effect of ROR-1 kringle domain on tumor cells was assessed using a proliferation assay with 1% bovine serum albumin (BSA) and 3 ng/mL bFGF in serum-free medium. Relative cell numbers in each well of a 96-well microplate after incubation

for 72 hours in the absence or presence of inhibitors were determined by using the AQueous Cell Proliferation Assay (Promega, Madison, WI). Duplicate plates with cells were also placed in hypoxia chambers with 95% N_2, 5%CO_2 at 37C for 72 hrs (Table 2). Results are presented as the percent inhibition of control cell (bFGF induced) proliferation. The ROR-1 kringle domain inhibited all sarcoma tumor cells in hypoxic conditions, but had no effect on the PC-3 prostate carcinoma cell line. These results indicate that ROR-1 is important for some tumor cell proliferation especially in stressed conditions.

Table 2. Effect of ROR-1 Kringle on proliferation in various cell lines.

		% Inhibition of Proliferation	
		normoxia	hypoxia
PC3	1000ng/ml	4(3)	0(8)
	100	0(9)	0(21)
sklms-1	1000ng/ml	0(1)	27(1)
sarcoma	100	0(4)	22(3)
HS883	1000ng/ml	0(4)	17(3)
sarcoma	100	0(8)	0(5)
HS913	1000ng/ml	0(3)	24(2)
sarcoma	100	0(2)	32(7)

CLAIMS

What is claimed is:

1. A method of determining whether a subject is a candidate for a therapy that comprises modulating a receptor tyrosine kinase orphan receptor-1 (ROR-1), said method comprising determining a level of ROR-1 in the subject, wherein the presence of an elevated level of ROR-1 indicates that the subject is a candidate for said therapy.

2. The method of claim 1, wherein the level of ROR-1 in the subject is determined by assaying a biological sample from the subject.

3. The method of claim 1, wherein the ROR-1 level comprises the level of ROR-1 polypeptide.

4. The method of claim 1, wherein the ROR-1 level comprises the ROR-1 expression level.

5. The method of claim 4, wherein ROR-1 genomic DNA copy number or methylation is measured as an indicator of ROR-1 expression level.

6. The method of claim 1, wherein the subject is in need of anti-angiogenesis therapy.

7. The method of claim 1, wherein the subject is in need of cancer therapy.

8. The method of claim 1, wherein the subject has, or is at risk, for a disease characterized by hypoxia.

9. The method of claim 1, wherein the subject has, or is at risk, for vascular disease.

10. The method of claim 1, wherein the subject has, or is at risk, for an inflammatory disease.

11. The method of claim 1, wherein the presence of ROR-1 at a level that is at least twice a control level indicates that the subject is a candidate for said therapy that comprises modulating a ROR-1.

12. The method of claim 1, additionally comprising determining a level of glucose-regulated protein 78 (GRP78) in the subject as an additional indicator of whether the subject is a candidate for said therapy, wherein the presence of an elevated level of GRP78 indicates that the subject is a candidate for said therapy.

13. The method of claim 12, wherein the level of GRP78 in the subject is determined by assaying a biological sample from the subject.

14. The method of claim 12, wherein the GRP78 level comprises the level of GRP78 polypeptide.

15. The method of claim 12, wherein the GRP78 level comprises the GRP78 expression level.

16. The method of claim 15, wherein GRP78 genomic DNA copy number or methylation is measured as an indicator of GRP78 expression level.

17. The method of claims 1 or 12, additionally comprising determining a level of a hypoxia indicator, wherein the presence of an elevated level of the hypoxia indicator indicates that the subject is a candidate for said therapy.

18. The method of claim 1, additionally comprising administering an agent that modulates ROR-1 to the subject.

19. The method of claim 1, wherein the agent is a peptide.

20. The method of claim 1, wherein the agent is an antibody.

21. The method of claim 1, wherein the agent is a small molecule.

22. The method of claim 1, wherein the agent inhibits the binding of ROR-1 to a membrane-bound protein involved in tumor survival.

23. The method of claim 22, wherein the agent inhibits the binding of ROR-1 to GRP78.

24. The method of claim 23, wherein the agent comprises K5 or a derivative thereof.

25. A method of determining whether a subject is responding to a therapy that comprises modulating a receptor tyrosine kinase orphan receptor-1 (ROR-1), said method comprising determining the level of ROR-1 in the subject at two different timepoints, wherein the first timepoint is before or during treatment and the second timepoint is during treatment and is later than the first timepoint, and wherein a decrease in ROR-1 level at the second timepoint, relative to the first, indicates that the subject is responding to said therapy.

26. The method of claim 25, wherein the level of ROR-1 in the subject is determined by assaying a biological sample from the subject.

27. The method of claim 25, wherein the ROR-1 level comprises the level of ROR-1 polypeptide.

28. The method of claim 25, wherein the ROR-1 level comprises the ROR-1 expression level.

29. The method of claim 25, wherein the therapy comprises anti-angiogenesis therapy.

30. The method of claim 25, wherein the therapy comprises cancer therapy.

31. The method of claim 25, wherein the subject has, or is at risk, for a disease characterized by hypoxia.

32. The method of claim 25, wherein the subject has, or is at risk, for vascular disease.

33. The method of claim 25, wherein the subject has, or is at risk, for an inflammatory disease.

34. The method of claim 25, additionally comprising determining a level of glucose-regulated protein 78 (GRP78) in the subject at said first and second timepoints as an additional indicator of whether the subject is responding to said therapy, wherein a decrease in GRP78 level at the second timepoint, relative to the first, indicates that the subject is responding to said therapy.

35. The method of claim 34, wherein the level of GRP78 in the subject is determined by assaying a biological sample from the subject.

36. The method of claims 25 or 34, additionally comprising determining a level of a hypoxia indicator in the subject at said first and second timepoints, wherein a decrease in the hypoxia indicator level at the second timepoint, relative to the first, indicates that the subject is responding to said therapy.

37. A method of imaging a region in the body of a subject, wherein the region is affected by a condition selected from the group consisting of angiogenesis, cancer, hypoxia, vascular disease, and inflammatory disease, the method comprising administering a labeled binding partner for a receptor tyrosine kinase orphan receptor-1 (ROR-1) to the subject, and detecting signal from the label.

38. A method of imaging a region in the body of of a subject, wherein the region is affected by a condition selected from the group consisting of angiogenesis, cancer, hypoxia, vascular disease, and inflammatory disease, the method comprising administering a labeled binding partner for a glucose-regulated protein 78 (GRP78) to the subject, and detecting signal from the label.

39. The method of claims 37 or 38, wherein the method comprises a method of imaging a tumor.

40. A method of testing a subject for the risk ,or presence, of a disease characterized by hypoxia, the method comprising determining a level of a receptor tyrosine kinase orphan receptor-1 (ROR-1) in the subject, wherein the presence of an

elevated level of ROR-1 indicates that the subject is at risk for, or has, a disease characterized by hypoxia.

41. A method of testing a subject for the risk ,or presence, of a disease characterized by hypoxia, the method comprising determining a level of glucose-regulated protein 78 (GRP78) in the subject, wherein the presence of an elevated level of GRP78 indicates that the subject is at risk for, or has, a disease characterized by hypoxia.

42. A method of modulating a receptor tyrosine kinase orphan receptor-1 (ROR-1), said method comprising contacting cells, at least some of which comprise ROR-1 and glucose-regulated protein 78 (GRP78), with an agent that inhibits the binding of ROR-1 to GRP78, provided that the agent is not a K5 peptide or a derivative thereof.

43. The method of claim 42, wherein the agent binds to a ROR-1 kringle domain.

44. The method of claim 42, wherein the agent is a competitive inhibitor of binding of a ROR-1 kringle domain to GRP78.

45. A method of modulating a receptor tyrosine kinase orphan receptor-1 (ROR-1) to produce an apoptotic effect, said method comprising contacting cells, at least some of which comprise ROR-1 and a Wnt, with an agent that inhibits binding of ROR-1 to Wnt, provided that the agent is not a K5 peptide or a derivative thereof.

46. The method of claim 45, wherein the agent binds to a ROR-1 frizzled domain.

47. The method of claim 45, wherein the agent is a competitive inhibitor of binding of a ROR-1 frizzled domain to Wnt.

48. The method of claim 45, wherein the Wnt is Wnt11.

49. The method of claim 45, wherein the Wnt is Wnt3.

50. A method of modulating a receptor tyrosine kinase orphan receptor-1 (ROR-1), said method comprising contacting cells, at least some of which comprise

ROR-1, with an agent that inhibits binding of the ROR-1 IgG domain to one or more ROR-1 binding partners, provided that the agent is not a K5 peptide or a derivative thereof.

51. The method of claim 50, wherein the agent binds to a ROR-1 IgG domain.

52. The method of claim 50, wherein the agent is a competitive inhibitor of binding of a ROR-1 IgG domain to said one or more ROR-1 binding partners.

53. A method of modulating a receptor tyrosine kinase orphan receptor-1 (ROR-1), said method comprising contacting cells, at least some of which comprise ROR-1, with an agent that inhibits binding of the ROR-1 kringle domain to one or more ROR-1 binding partners, provided that the agent is not a K5 peptide or a derivative thereof.

54. The method of claim 53, wherein the agent binds to a ROR-1 kringle domain.

55. The method of claim 53, wherein the agent is a competitive inhibitor of binding of a ROR-1 kringle domain to said one or more ROR-1 binding partners.

56. The method of claim 42, wherein the agent is a peptide.

57. The method of claim 42, wherein the agent is an antibody.

58. The method of claim 42, wherein the agent is a small molecule.

59. The method of claim 42, wherein the method of modulating a ROR-1 produces an anti-angiogenic effect.

60. The method of claim 42, wherein the method of modulating a ROR-1 produces an apoptotic effect.

61. The method of claim 42, wherein the cells comprise endothelial cells.

62. The method of claim 42, wherein the cells comprise cancer cells.

63. The method claim 42, wherein the cells comprise cells subject to a stress selected from the group consisting of a hypoxic stress or a chemical stress.

64. The method of claim 42, wherein the cells are in vitro.

65. The method of claim 42, wherein the cells are in vivo.

66. The method of claim 65, wherein cells are present in a subject in need of anti-angiogenesis therapy.

67. The method of claim 65, wherein cells are present in a subject in need of cancer therapy.

68. The method of claim 67, wherein the method additionally comprises administering a cancer chemotherapeutic agent to the subject.

69. An antibody specific for ROR-1, wherein the antibody inhibits the binding of ROR-1 to GRP78 and is not a polyclonal antibody.

70. The antibody of claim 69, wherein the antibody binds to an epitope within a ROR-1 kringle domain.

71. The antibody of claim 70, wherein the antibody is produced by a process comprising immunizing an animal with a ROR-1 kringle domain or a derivative thereof and/or by a process comprising screening monoclonal antibodies or Fab fragments for binding to a ROR-1 kringle domain or a derivative thereof.

72. An antibody specific for GRP78, wherein the antibody inhibits the binding of GRP78 to ROR-1.

73. An antibody specific for ROR-1, wherein the antibody inhibits binding of ROR-1 to a Wnt and is not a polyclonal antibody.

74. The antibody of claim 73, wherein the antibody binds to an epitope within a ROR-1 frizzled domain.

75. The antibody of claim 74, wherein the antibody is produced by a process comprising immunizing an animal with the ROR-1 frizzled domain or a derivative thereof and/or by a process comprising screening monoclonal antibodies or Fab fragments for binding to the ROR-1 frizzled domain or a derivative thereof.

76. The antibody of claim 73, wherein the Wnt is Wnt11.

77. The antibody of claim 73, wherein the Wnt is Wnt3.

78. An antibody specific for Wnt11, wherein the antibody inhibits binding of Wnt11 to ROR-1.

79. An antibody specific for Wnt3, wherein the antibody inhibits binding of Wnt3 to ROR-1.

80. An antibody that specifically binds the complex of receptor tyrosine kinase orphan receptor-1 (ROR-1) with GRP78, wherein the antibody is not a polyclonal antibody.

81. An antibody that specifically binds the complex of receptor tyrosine kinase orphan receptor-1 (ROR-1) with a Wnt, wherein the antibody is not a polyclonal antibody.

82. The antibody of claim 81, wherein the Wnt is Wnt11.

83. The antibody of claim 81, wherein the Wnt is Wnt3.

84. A method of screening for an agent that modulates receptor tyrosine kinase orphan receptor-1 (ROR-1), the method comprising:

a) contacting a test agent with a ROR-1 polypeptide in the presence of a GRP78 polypeptide; and

b) detecting specific binding of the ROR-1 polypeptide with the GRP78 polypeptide relative to the said specific binding in the absence of the test agent or in the presence of a lower amount of test agent.

85. The method of claim 84, wherein any test agent that inhibits specific binding of the ROR-1 polypeptide with the GRP78 polypeptide is selected as an inhibitor of ROR-1.

86. A method of prescreening for an agent that modulates angiogenesis and/or apoptosis, the method comprising:

a) contacting a test agent with a receptor tyrosine kinase orphan receptor-1 (ROR-1) polypeptide or polynucleotide; and

b) detecting specific binding of the test agent to the ROR-1 polypeptide or polynucleotide.

87. A method of screening for an agent that modulates angiogenesis and/or apoptosis, the method comprising:

a) contacting a test agent with a cell that expresses a ROR-1 polypeptide; and

b) determining the level of:

(i) ROR-1 polypeptide; or

(ii) ROR-1 RNA,

relative to the level in a cell that has not been contacted with the test agent or has been contacted with a lower amount of test agent.

88. The method of claim 87, additionally comprising selecting any test agent that reduces said level as a modulator of angiogenesis.

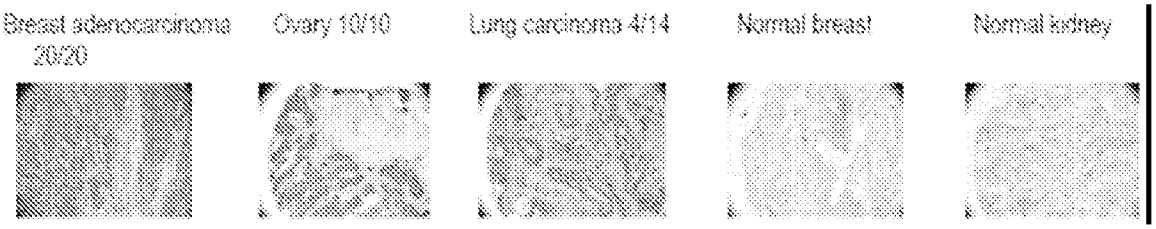
Breast adenocarcinoma 20/20
Ovary 10/10
Lung carcinoma 4/14
Normal breast
Normal kidney

Fig. 1

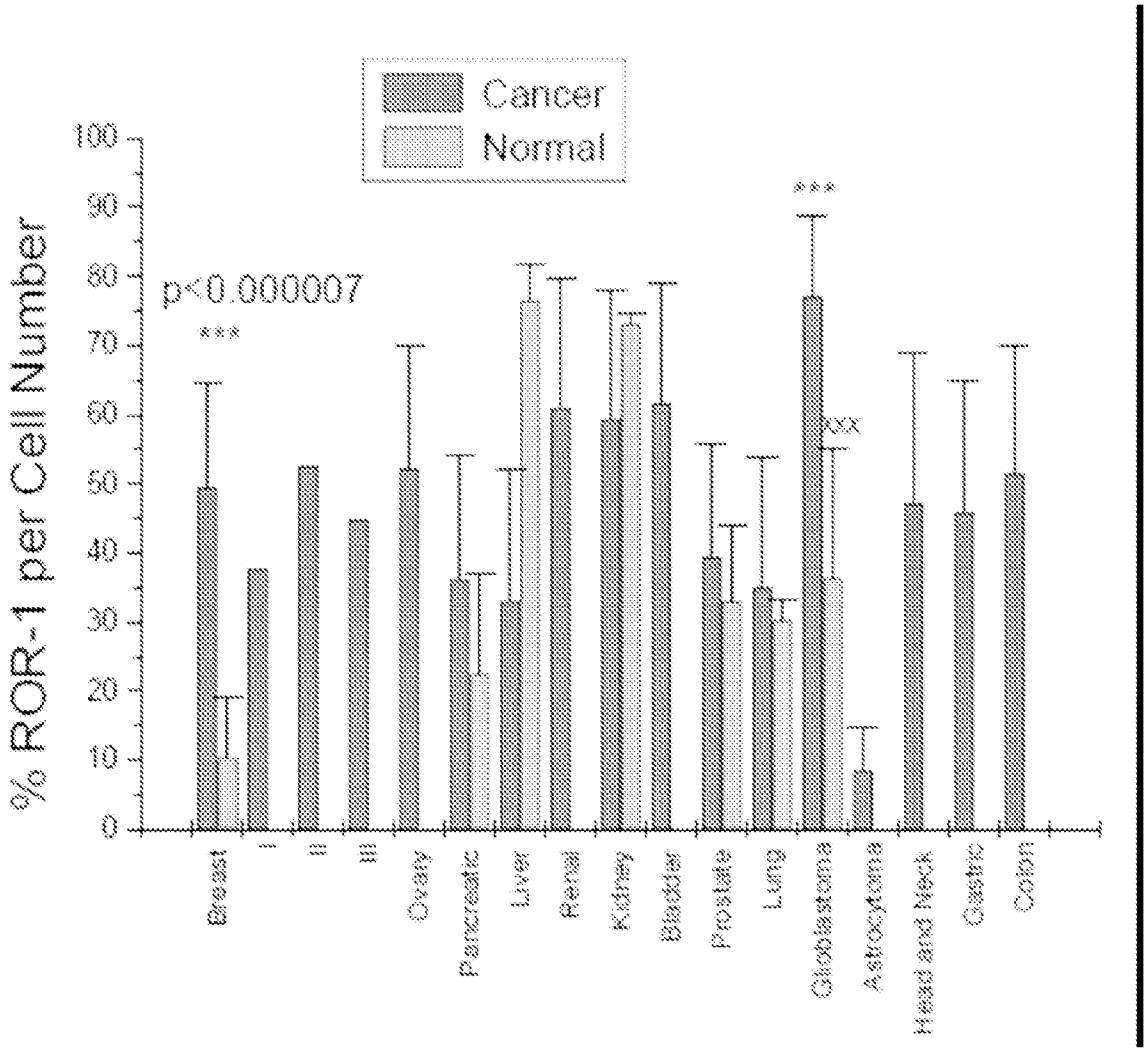
Cancer
Normal
% ROR-1 per Cell Number
100
90
80
70
60
50
40
30
20
10
0
p<0.000007
***
***
xxx
Breast
I
II
III
Ovary
Pancreatic
Liver
Renal
Kidney
Bladder
Prostate
Lung
Glioblastoma
Astrocytoma
Head and Neck
Gastric
Colon

Fig. 2

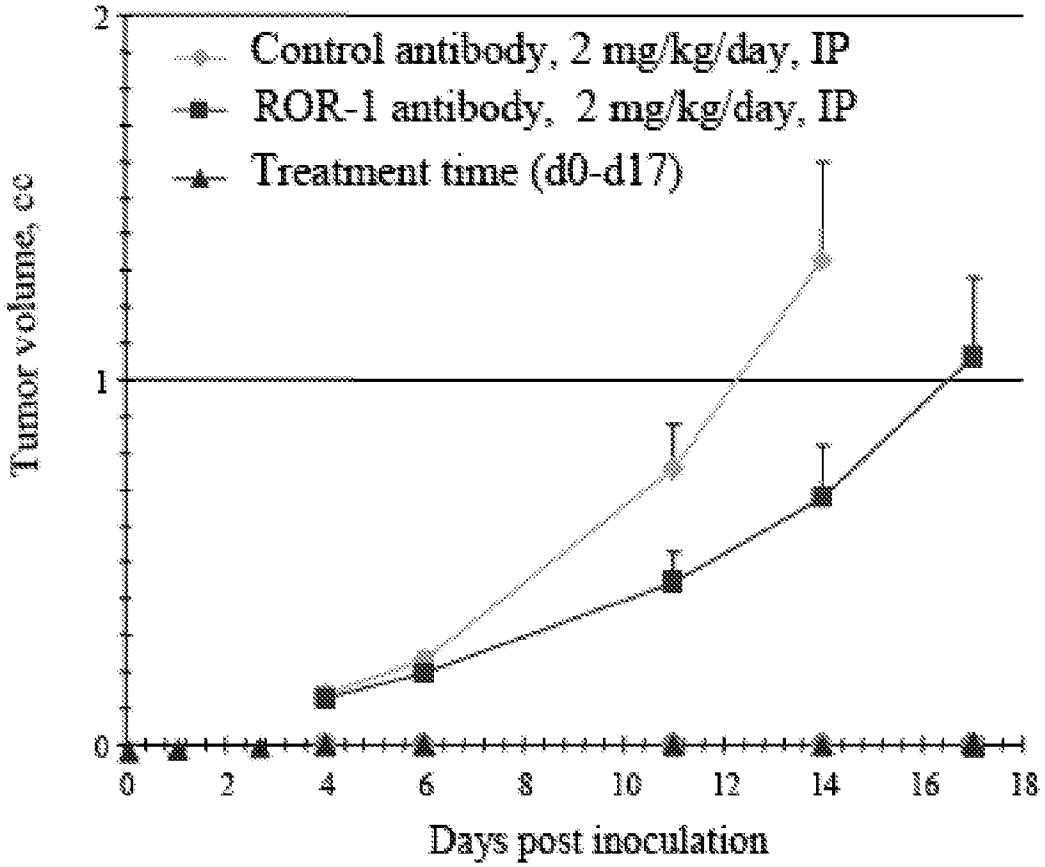
Control antibody, 2 mg/kg/day, IP
ROR-1 antibody, 2 mg/kg/day, IP
Treatment time (d0-d17)
Tumor volume, cc
2
1
0
0
2
4
6
8
10
12
14
16
18
Days post inoculation

Fig. 3

Ror-1 domains translated ref_seq NM_005012 (SEQ ID NO: 1)

Ror Ig (SEQ ID NO: 2)

Ror Kringle Domain (SEQ ID NO: 3)

1-50

Ror-1 (1) MHRPRRRGTRPPLLALLAALLLAARGAAAQETELSVSAELVPTSSWNISS

Ror Ig (1) ----------MGSSHHHHHHSS

51 100

Ror-1 (51) ELNKDSYLTLDEPMNNITTSLGQTAELHCKVSGNPPPTIRWFKNDAPVVQ

Ror Ig (13)GLVPRGSHMLDEPMNNITTSLGQTAELHCKVSGNPPPTIRWFKNDAPVVQ

101 150

Ror-1(101) EPRRLSFRSTIYGSRLRIRNLDTTDTGYFQCVATNGKEVVSSTGVLFVKF

Ror Ig (63) EPRRLSFRSTIYGSRLRIRNLDTTDTGYFQCVATNGKEVVSSTGVL----------

151 200

Ror-1 (151) GPPPTASPGYSDEYEEDGFCQPYRGIACARFIGNRTVYMESLHMQGEIEN

201 250

Ror-1 (201) QITAAFTMIGTSSHLSDKCSQFAIPSLCHYAFPYCDETSSVPKPRDLCRD

251 300

Ror-1 (251) ECEILENVLCQTEYIFARSNPMILMRLKLPNCEDLPQPESPEAANCIRIG

Ror Kr (1) ----------MGSSHHHHHHSS

301 350

Ror-1 (301) IPMADPINKNHKCYNSTGVDYRGTVSVTKSGRQCQPWNSQYPHTHTFTAL

Ror Kr (13) GLVPRGSHMNHKCYNSTGVDYRGTVSVTKSGRQCQPWNSQYPHTHTFTAL

351 400

Ror-1 (351) RFPELNGGHSYCRNPGNQKEAPWCFTLDENFKSDLCDIPACDSKDSKEKN

Ror Kr (63) RFPELNGGHSYCRNPGNQKEAPWCFTLDENFKSDLCDIPACD--------

401 450

Ror-1 (401) KMEILYILVPSVAIPLAIALLFFFICVCRNNQKSSSAPVQRQPKHVRGQN

451 500

Ror-1 (451) VEMSMLNAYKPKSKAKELPLSAVRFMEELGECAFGKIYKGHLYLPGMDHA

501 550

Ror-1 (501) QLVAIKTLKDYNNPQQWMEFQQEASLMAELHHPNIVCLLGAVTQEQPVCM

551 600

Ror-1 (551) LFEYINQGDLHEFLIMRSPHSDVGCSSDEDGTVKSSLDHGDFLHIAIQIA

601 650

Ror-1 (601) AGMEYLSSHFFVHKDLAARNILIGEQLHVKISDLGLSREIYSADYYRVQS

651 700

Ror-1 (651) KSLLPIRWMPPEAIMYGKFSSDSDIWSFGVVLWEIFSFGLQPYYGFSNQE

701 750

Ror-1 (701) VIEMVRKRQLLPCSEDCPPRMYSLMTECWNEIPSRRPRFKDIHVRLRSWE

751 800

Ror-1 (751) GLSSHTSSTTPSGGNATTQTTSLSASPVSNLSNPRYPNYMFPSQGITPQG

801 850

Ror-1 NM_005012 (801) QIAGFIGPPIPQNQRFIPINGYPIPPGYAAFPAAHYQPTGPPRVIQHCPP

851 900

Ror-1 (851) PKSRSPSSASGSTSTGHVTSLPSSGSNQEANIPLLPHMSIPNHPGGMGIT

901 938

Ror-1 (901) VFGNKSQKPYKIDSKQASLLGDANIHGHTESMISAEL-

Fig. 4B

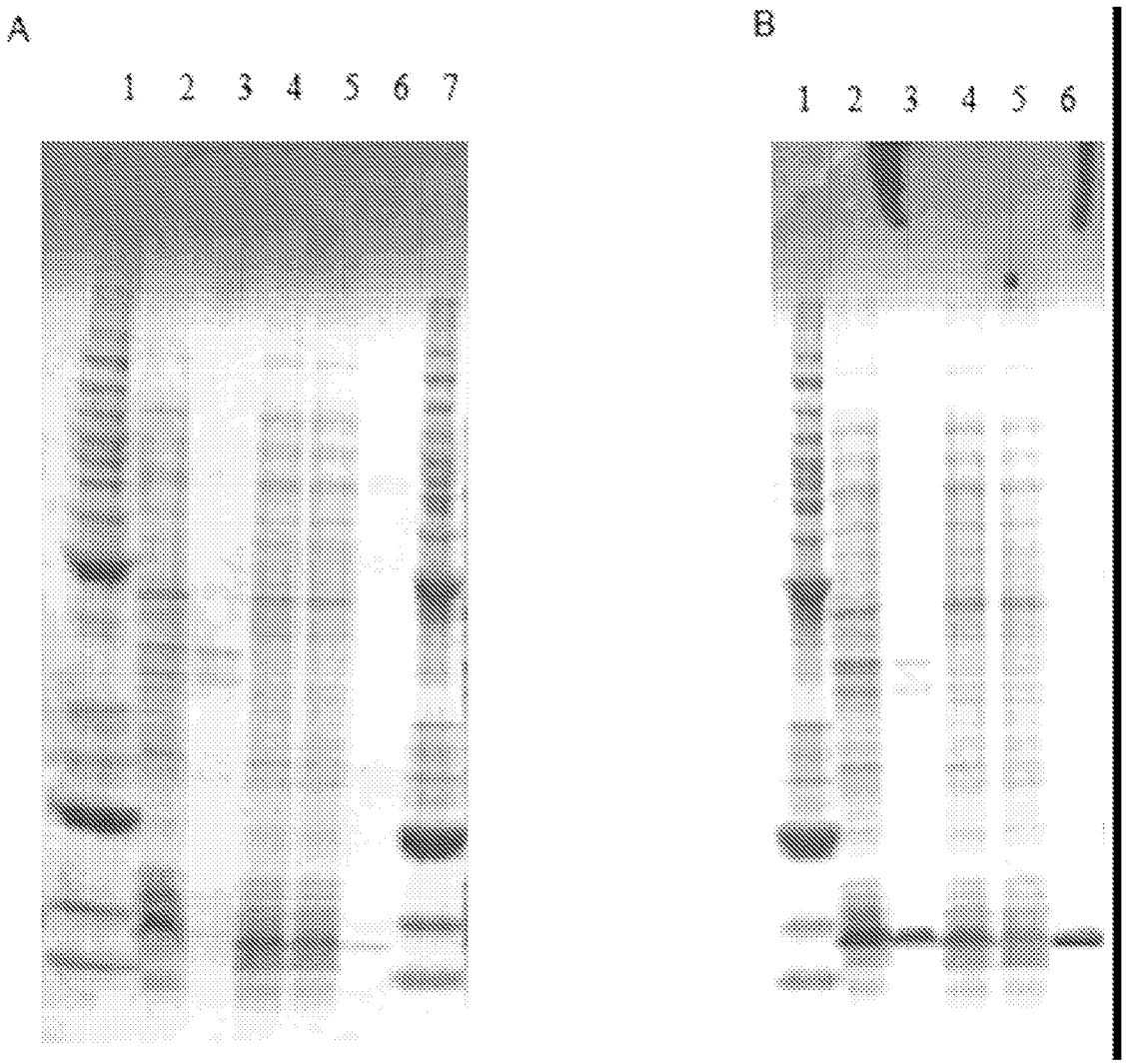

Fig. 5

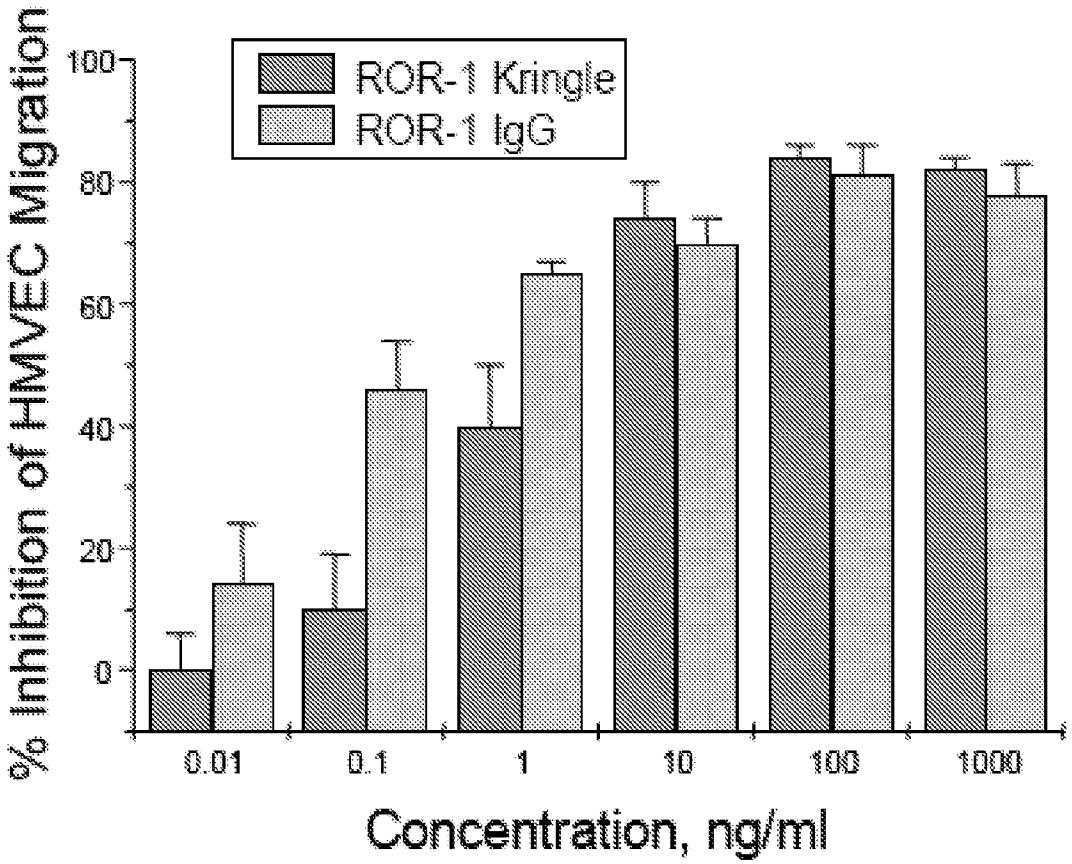
% Inhibition of HMVEC Migration
100
80
60
40
20
0
ROR-1 Kringle
ROR-1 IgG
0.01
0.1
1
10
100
1000
Concentration, ng/ml

Fig. 6

```
GLU PRO LEU ASP ASP TYR VAL ASN THR GLN GLY ALA SER LEU PHE
1               5                  10                  15

SER VAL THR LYS LYS GLN LEU GLY ALA GLY SER ILE GLU GLU CYS
            20                  25                  30

ALA ALA LYS CYS GLU GLU ASP GLU GLU PHE THR CYS ARG ALA PHE
        35                  40                  45

GLN TYR HIS SER LYS GLU GLN GLN CYS VAL ILE MET ALA GLU ASN
    50                  55                  60

ARG LYS SER SER ILE ILE ILE ARG MET ARG ASP VAL VAL LEU PHE
65                  70                  75

GLU LYS LYS VAL TYR LEU SER GLU CYS LYS THR GLY ASN GLY LYS
            80                  85                  90

ASN TYR ARG GLY THR MET SER LYS THR LYS ASN GLY ILE THR CYS
        95                  100                 105

GLN LYS TRP SER SER THR SER PRO HIS ARG PRO ARG PHE SER PRO
    110                 115                 120

ALA THR HIS PRO SER GLU GLY LEU GLU GLU ASN TYR CYS ARG ASN
125                 130                 135

PRO ASP ASN ASP PRO GLN GLY PRO TRP CYS TYR THR THR ASP PRO
            140                 145                 150

GLU LYS ARG TYR ASP TYR CYS ASP ILE LEU GLU CYS GLU GLU GLU
        155                 160                 165

CYS MET HIS CYS SER GLY GLU ASN TYR ASP GLY LYS ILE SER LYS
    170                 175                 180

THR MET SER GLY LEU GLU CYS GLN ALA TRP ASP SER GLN SER PRO
185                 190                 195

HIS ALA HIS GLY TYR ILE PRO SER LYS PHE PRO ASN LYS ASN LEU
            200                 205                 210

LYS LYS ASN TYR CYS ARG ASN PRO ASP ARG GLU LEU ARG PRO TRP
        215                 220                 225

CYS PHE THR THR ASP PRO ASN LYS ARG TRP GLU LEU CYS ASP ILE
    230                 235                 240

PRO ARG CYS THR THR PRO PRO PRO SER SER GLY PRO THR TYR GLN
245                 250                 255

CYS LEU LYS GLY THR GLY GLU ASN TYR ARG GLY ASN VAL ALA VAL
            260                 265                 270
```

Fig. 7A

```
THR VAL SER GLY HIS THR CYS GLN HIS TRP SER ALA GLN THR PRO
                275                 280                 285

HIS THR HIS ASN ARG THR PRO GLU ASN PHE PRO CYS LYS ASN LEU
            290                 295                 300

ASP GLU ASN TYR CYS ARG ASN PRO ASP GLY LYS ARG ALA PRO TRP
        305                 310                 315

CYS HIS THR THR ASN SER GLN VAL ARG TRP GLU TYR CYS LYS ILE
    320                 325                 330

PRO SER CYS ASP SER SER PRO VAL SER THR GLU GLN LEU ALA PRO
335                 340                 345

THR ALA PRO PRO GLU LEU THR PRO VAL VAL GLN ASP CYS TYR HIS
            350                 355                 360

GLY ASP GLY GLN SER TYR ARG GLY THR SER SER THR THR THR THR
        365                 370                 375

GLY LYS LYS CYS GLN SER TRP SER SER MET THR PRO HIS ARG HIS
    380                 385                 390

GLN LYS THR PRO GLU ASN TYR PRO ASN ALA GLY LEU THR MET ASN
395                 400                 405

TYR CYS ARG ASN PRO ASP ALA ASP LYS GLY PRO TRP CYS PHE THR
            410                 415                 420

THR ASP PRO SER VAL ARG TRP GLU TYR CYS ASN LEU LYS LYS CYS
        425                 430                 435

SER GLY THR GLU ALA SER VAL VAL ALA PRO PRO PRO VAL VAL LEU
    440                 445                 450

LEU PRO ASP VAL GLU THR PRO SER GLU GLU ASP CYS MET PHE GLY
455                 460                 465

ASN GLY LYS GLY TYR ARG GLY LYS ARG ALA THR THR VAL THR GLY
            470                 475                 480

THR PRO CYS GLN ASP TRP ALA ALA GLN GLU PRO HIS ARG HIS SER
        485                 490                 495

ILE PHE THR PRO GLU THR ASN PRO ARG ALA GLY LEU GLU LYS ASN
    500                 505                 510

TYR CYS ARG ASN PRO ASP GLY ASP VAL GLY GLY PRO TRP CYS TYR
515                 520                 525

THR THR ASN PRO ARG LYS LEU TYR ASP TYR CYS ASP VAL PRO GLN
            530                 535                 540
```

Fig. 7B

CYS ALA ALA PRO SER PHE ASP CYS GLY LYS PRO GLN VAL GLU PRO
545 550 555

LYS LYS CYS PRO GLY ARG VAL VAL GLY GLY CYS VAL ALA HIS PRO
560 565 570

HIS SER TRP PRO TRP GLN VAL SER LEU ARG THR ARG PHE GLY MET
575 580 585

HIS PHE CYS GLY GLY THR LEU ILE SER PRO GLU TRP VAL LEU THR
590 595 600

ALA ALA HIS CYS LEU GLU LYS SER PRO ARG PRO SER SER TYR LYS
605 610 615

VAL ILE LEU GLY ALA HIS GLN GLU VAL ASN LEU GLU PRO HIS VAL
620 625 630

GLN GLU ILE GLU VAL SER ARG LEU PHE LEU GLU PRO THR ARG LYS
635 640 645

ASP ILE ALA LEU LEU LYS LEU SER SER PRO ALA VAL ILE THR ASP
650 655 660

LYS VAL ILE PRO ALA CYS LEU PRO SER PRO ASN TYR VAL VAL ALA
665 670 675

ASP ARG THR GLU CYS PHE ILE THR GLY TRP GLY GLU THR GLN GLY
680 685 690

THR PHE GLY ALA GLY LEU LEU LYS GLU ALA GLN LEU PRO VAL ILE
695 700 705

GLU ASN LYS VAL CYS ASN ARG TYR GLU PHE LEU ASN GLY ARG VAL
710 715 720

GLN SER THR GLU LEU CYS ALA GLY HIS LEU ALA GLY GLY THR ASP
725 730 735

SER CYS GLN GLY ASP SER GLY GLY PRO LEU VAL CYS PHE GLU LYS
740 745 750

ASP LYS TYR ILE LEU GLN GLY VAL THR SER TRP GLY LEU GLY CYS
755 760 765

ALA ARG PRO ASN LYS PRO GLY VAL TYR VAL ARG VAL SER ARG PHE
770 775 780

VAL THR TRP ILE GLU GLY VAL MET ARG ASN ASN
785 790

Fig. 7C